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Full Name of Author — Nom complet de l'auteur
WARREN BERT KINDZIERSKI

Date of Birth — Date de naissance
MAY 15, 1957

Country of Birth — Lieu de naissance
CANADA

Permanent Address — Résidence fixe
BOX 276
ARBORG MANITOBA
R0C 0A0

Title of Thesis — Titre de la thèse
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1984

Name of Supervisor — Nom du directeur de thèse
STEVE E. HRUDEY

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EFFECTS OF PHOSPHORUS REMOVAL CHEMICALS UPON ANAEROBIC SLUDGE DIGESTION

by

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A THESIS
SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH
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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research, for acceptance, a thesis entitled EFFECTS OF PHOSPHORUS REMOVAL CHEMICALS UPON ANAEROBIC SLUDGE DIGESTION submitted by WARREN B. KINDZIERSKI in partial fulfilment of the requirements for the degree of MASTER OF SCIENCE.

....
Supervisor

Daniel W. Smith

P Fedorak

Date July 12, 1984

ABSTRACT

Alum and ferric chloride are commonly employed to aid phosphorus removal in wastewater treatment. Previous studies have indicated the use of these chemical coagulants produce sludges that adversely affect anaerobic digestion. The objective of this research was to assess the magnitude of effects chemical coagulants have upon anaerobic digestion by monitoring methane production and measuring concentrations of aluminum or iron present during batch digestion of chemically precipitated sludge.

Alum addition to wastewater produced sludge that demonstrated reduced methane production when batch digested anaerobically. The magnitude of the adverse effects on methane production increased with increasing aluminum concentrations up to 144 mg/L. The type or nature of chemical floc that forms during the actual coagulation process may play an important role in influencing the magnitude of the adverse effects during digestion. The nature of the chemical effect appeared to limit the extent or degree to which the anaerobic microorganisms were able to metabolize the organic wastes and appeared to occur with the non-methanogenic species.

Ferric chloride addition to wastewater produced sludge that, when batch digested anaerobically, demonstrated reduced methane production, however the magnitude of the adverse effects did not increase with increasing iron concentrations up to 770 mg/L. The nature of the chemical

effect appeared to occur with the non-methanogenic species.

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1. INTRODUCTION

The use of chemical coagulants such as alum or ferric chloride in wastewater treatment can be a practical method for improving suspended solids and/or phosphorus removal. Chemical phosphorus removal is employed at Calgary, Alberta to reduce phosphorus input from wastewater discharges into the Bow River. Although effective phosphorus removal can be achieved, the existing biological treatment plant can experience considerable changes in overall process performance. The effect of phorphorus removal chemicals upon anaerobic sludge digestion is of particular interest in this respect.

When chemically precipitated sludges are fed to anaerobic digesters, reduced digestion performance can occur due to an increased stress associated with higher organic loadings or lower feed sludge pH (Emig, 1979; Shannon et al., 1974). Studies by Gossett et al. (1979), on the other hand , have indicated chemical coagulation of domestic wastewater affects digestibility of the resulting sludges. Reduced digestion performance has been demonstrated by decreased total gas production, methane production, and volatile solids destruction. It was proposed these effects are apparently attributed to a mechanism that renders volatile solids less accessible and/or less reactive to extracellular enzymes of the acid forming bacteria during the digestion process.

The literature has demonstrated the adverse effects of alum and ferric chloride upon anaerobic digestion. However, there exists a lack of information in these studies on chemical coagulant concentrations present during digestion which produce the adverse effects.

The objective of this research was to assess the magnitude of effects chemical coagulants have upon anaerobic digestion by monitoring methane production and measuring concentrations of aluminum or iron present during batch digestion of chemically precipitated sludge. More specifically:

1. to determine if a chemical coagulant dose methane response relationship exists in order to establish threshold values for any adverse effects which may be caused by alum or ferric chloride; and
2. to determine any relationship between the experimental results and digester performance at a full scale plant where chemical coagulant addition is practiced for phosphorus removal.

2. BASIC CONSIDERATIONS FOR PHOSPHORUS REMOVAL

The primary sources of phosphorus in domestic wastewater are a result of man's activities in the home (EPA,1976). Human wastes and waste food disposal can account for 30 to 50 percent of the phosphorus loading. Phosphate builders used in detergents can account for the remaining 50 to 70 percent. Other sources can originate from phosphorus compounds used as corrosion and scale control chemicals in water supplies and industrial wastewater discharges (e.g. potato processing plants). These sources can account for 2 to 20 percent of total phosphorus present in wastewater.

Phosphorus, when present in excess, can create pollution problems in receiving streams by causing excess growth of algae and rooted aquatic plants. Subsequent problems that may arise in receiving streams include (WPCF, 1977):

1. dissolved oxygen levels can be reduced;
2. growth of game fish may be discouraged;
3. odor problems can occur; and
4. recreational use may become undesirable.

Raw wastewaters typically contain 3 to 15 mg/L of phosphorus (as P)(Metcalf and Eddy, 1979). Influent phosphorus levels of 3 to 5 mg/L (as P) in raw wastewater are successfully reduced to 0.5 mg/L (as P) in the plant effluent by alum addition at the City of Calgary Bonnybrook Wastewater Treatment Plant (WWTP)(Interview dated 1983

November 23 with Bob Mackintosh, Bonnybrook WWTP, Calgary Alberta).

3. PHOSPHORUS REMOVAL CHEMICALS IN ANAEROBIC SLUDGE DIGESTION

3.1 Chemistry of Phosphorus Removal

Phosphorus is found in three principal forms:

1. orthophosphates (soluble inorganics);
2. polyphosphates (soluble inorganics); and
3. organic phosphorus compounds.

All polyphosphates gradually hydrolyze in aqueous solution and revert to orthophosphates (the form from which they were derived) (Sawyer and McCarty, 1980). Prolonged contact with microorganisms in raw wastewater and secondary biological treatment ensures this reversion (Snoeyink and Jenkins, 1980). Organic compounds decompose and their phosphorus content is converted to orthophosphates during biological treatment. Some inorganic phosphates are utilized in the formation of biological floc, however the inorganic phosphorus content in wastewater is in excess of nutrient requirements for aerobic biological treatment (Metcalf and Eddy, 1979). A well treated secondary effluent contains a large fraction of the phosphorus present as orthophosphates. This is fortunate since orthophosphates are the easiest form to precipitate by chemical treatment.

3.1.1 Aluminum Phosphate Precipitation

Aluminum ions can combine with orthophosphate ions to form aluminum phosphate as follows (Metcalf and Eddy, 1979):

$$Al^{3+} + H_nPO_4^{3-n} \leftrightarrow AlPO_4\downarrow + nH^+.$$

The most common source of aluminum for use in phosphorus precipitation is alum:

$$Al_2(SO_4)_3 \cdot 18H_2O + 2PO_4^{3-} \leftrightarrow 2AlPO_4\downarrow + 3SO_4^{2-} + 18H_2O.$$

The solubility of aluminum phosphate is a function of pH (Table 3.1). Optimum pH for phosphorus removal lies in the range of 5.5 to 6.5.

Alum will react with alkalinity present in wastewater (WPCF, 1977):

$$Al_2(SO_4)_3 \cdot 18H_2O + 6HCO_3^- \leftrightarrow 2Al(OH)_3\downarrow + 3SO_4^{2-} + 6CO_2\uparrow + 18\,H_2O.$$

This hydrolyzing reaction competes with the foregoing phosphate reaction for available alum. For this reason, laboratory jar tests best determine the chemical dosages required to achieve acceptable phosphorus removal. Snoeyink and Jenkins (1980) note that the aluminum hydroxide precipitate is important because it aids in the sedimentation of the aluminum phosphate precipitate. Two mechanisms by which this is possible are (Snoeyink and Jenkins, 1980; WPCF, 1977):

1. the well flocculating aluminum hydroxide precipitate sweeps out the colloidal, rather difficult to settle, aluminum phosphate precipitate; and
2. phosphate adsorption onto or incorporation into the aluminum hydroxide precipitate can occur.

Addition of alum will lower the pH of wastewater because of neutralization of alkalinity and release of

Table 3.1 Solubility of Aluminum Phosphate Versus pH
(After WPCF, 1977)

pH	Approximate Solubility $AlPO_4$ (mg/L)
5	0.03
6	0.01
7	0.30

carbon dioxide. In cases where the natural alkalinity of wastewater is inadequate for the alum dosage, pH reduction may be so great that addition of an alkaline substance is required (EPA, 1976).

3.1.2 Iron Phosphate Precipitation

Ferric ions can combine with orthophosphate ions to form ferric phosphate as follows (Metcalf and Eddy, 1979):

$$Fe^{3+} + H_nPO_4^{3-n} \leftrightarrow FePO_4\downarrow + nH^+.$$

Ferric chloride is commonly used to precipitate phosphorus:

$$FeCl_3 + PO_4^{3-} \leftrightarrow FePO_4\downarrow + 3Cl^-.$$

The optimun pH range for iron precipitation of phosphorus is 4.5 to 5.0 (EPA, 1976).

Ferric chloride also reacts with alkalinity present in wastewater (WPCF, 1977):

$$2FeCl_3 + 6HCO_3^- \leftrightarrow 2Fe(OH)_3\downarrow + 6Cl^- + 6CO_2\uparrow.$$

As in the case with alum, the hydrolyzing reaction competes with the phosphate reaction for available ferric chloride. The ferric hydroxide percipitate aids removal of the ferric phosphate precipitate (WPCF, 1977). Alkalinity adjustments to wastewater containing low natural alkalinity, discussed previously, apply for iron phosphate precipitation.

3.2 Anaerobic Digestion Theory

Anaerobic digestion is the biological degradation of complex organic substances in the absence of oxygen (EPA, 1979). During the process, much of the organic matter is converted to methane, carbon dioxide, and water. Since the process does not proceed completely to end products of carbon dioxide and water (lowest energy compounds in biological degradation), there is energy retained as methane. The remaining solids are rendered stable as little carbon and energy remain available to sustain further biological activity. Approximately 70% of total gas production is in the form of methane.

3.2.1 Anaerobic Digestion Process

Anaerobic degradation of complex organic substances is accomplished by the combined and coordinated metabolic activity of digester bacteria. Figure 3.1 summarizes the four groups of bacteria essential to the digestion process.

The consortium of bacteria consist of (Mosey, 1982; Gaudy and Gaudy, 1980):

1. Acid Forming Bacteria (these hydrolytic bacteria ferment complex organics to produce organic acids and neutral compounds);
2. Acetogenic Bacteria (this group of bacteria ferments long chain organic acids, other than acetic acid, to end products of acetic acid and neutral compounds);
3. Acetate Methanogenic Bacteria (these bacteria utilize acetic acid to account for approximately 75 percent of the methane formed in sludge digestion); and

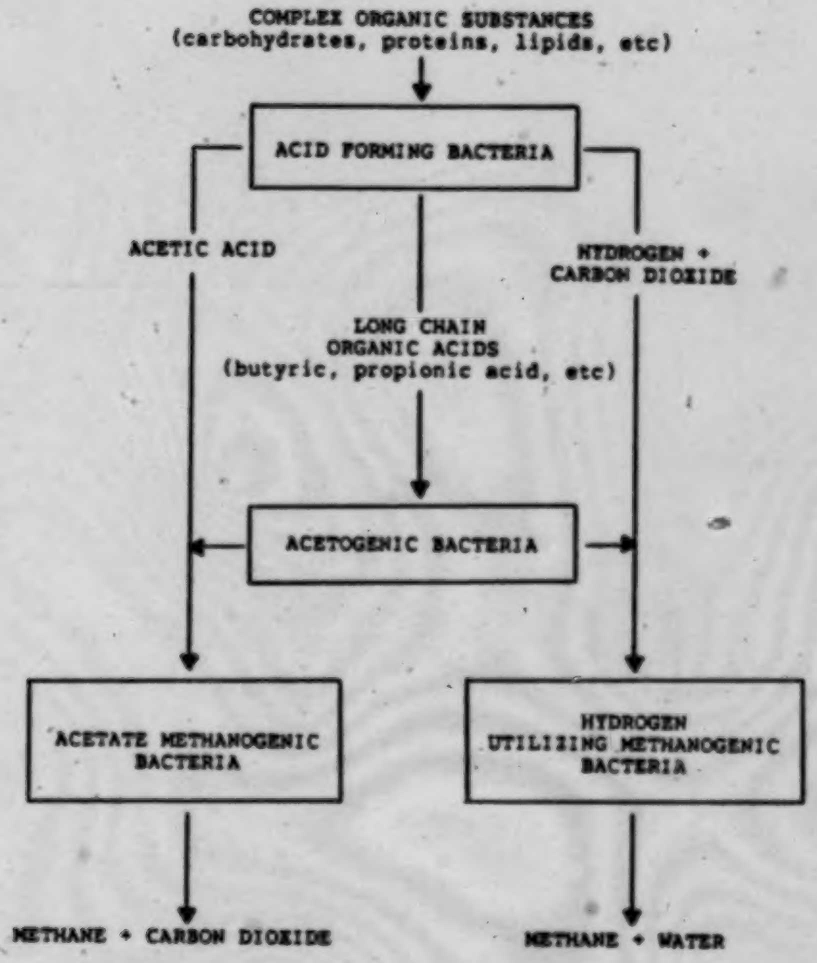
COMPLEX ORGANIC SUBSTANCES
(carbohydrates, proteins, lipids, etc)
ACID FORMING BACTERIA
ACETIC ACID
HYDROGEN + CARBON DIOXIDE
LONG CHAIN ORGANIC ACIDS
(butyric, propionic acid, etc)
ACETOGENIC BACTERIA
ACETATE METHANOGENIC BACTERIA
HYDROGEN UTILIZING METHANOGENIC BACTERIA
METHANE + CARBON DIOXIDE
METHANE + WATER

Figure 3.1 Microbiology of Anaerobic Digestion
(Adapted from Mosey, 1982)

4. Hydrogen Utilizing Methanogenic Bacteria (these bacteria convert hydrogen and carbon dioxide to contribute the remaining 25 percent methane production).

3.2.2 Environmental Requirements

Growth rates for acetate methanogens are relatively slow (minimum doubling times of 2 to 3 days at 35 °C) and that of the acid formers is potentially fast (minimum doubling times of 2 to 3 hours at 35 °C) under optimum conditions. Suitable environmental conditions are, therefore, important to ensure successful operation of this complex microbiological system.

3.2.2.1 pH and Alkalinity

Close pH control is necessary as methanogenic bacteria exhibit sensitivity to pH changes outside the range of 6.5 to 7.5 (EPA, 1979). Within this range the carbon dioxide-bicarbonate alkalinity system governs the pH. Gossett et al. (1978) indicated that alkalinity in domestic digested sludge results mainly from ammonia, released by degradation of organic nitrogen materials (protein and urea). Ammonia in the presence of carbon dioxide and water will form ammonium bicarbonate:

$$NH_3 + CO_2 \leftrightarrow HCO_3^- + NH_4^+.$$

Bicarbonate alkalinity will be destroyed as organic acids accumulate in the system (Sawyer and McCarty, 1980):

$$R\text{-}COOH + HCO_3^- \leftrightarrow R\text{-}COO^- + CO_2 + H_2O.$$

The alkalinity acts in a buffering capacity to lessen changes in pH. Figure 3.2 indicates the limits of normal anaerobic treatment for an operating temperature of 35 °C. Bicarbonate alkalinity in the range of 2,500 to 5,000 mg/L (as $CaCO_3$) is desirable to provide adequate buffering capacity so that large increases in organic acids can be tolerated with minimal pH change.

3.2.2.2 Temperature and Solids Retention Time

Anaerobic digestion can take place at any temperature between 5 to 55 °C. The rate of total gas production increases with temperature (Meynell, 1982), however digesters seldom operate at the upper range because of increased energy requirements to maintain the higher temperature. Adequate solids retention time is required to give acceptable degradation of organic matter, satisfactory gas production, and sufficient reduction of pathogens. Table 3.2 shows minimum and design solids retention times for a range of operating temperatures for high rate digestion. Meynell (1982) indicated an optimum temperature range of 30 to 35 °C provides the best conditions for bacterial growth and methane production.

3.2.2.3 Toxic Materials

For a material to be biologically toxic it must be in solution. Table 3.3 lists stimulatory and inhibitory

Table 3.2 Solids Retention Times For Complete-Mix Digesters
(After WPCF, 1977)

Operating Temperature (°C)	Solids Retention Time (Days) Minimum	 Suggested For Design
10	-	55
20	28	40
25	20	30
30	14	25
35	10	20
40	10	20
45	-	15

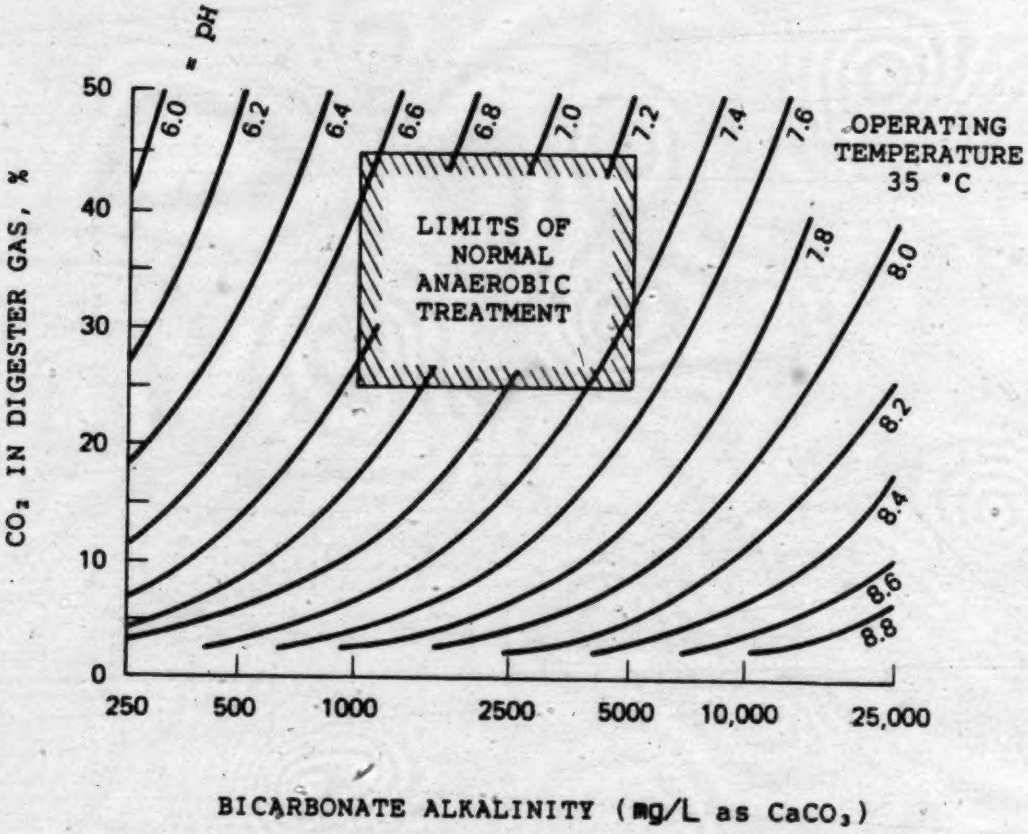

Figure 3.2 Relationship Between pH and Bicarbonate Alkalinity (After McCarty, 1964a)

concentrations of potentially toxic materials.

Ammonia may be present in either the form of ammonium ion or as a dissolved gas depending upon the pH and temperature:

$NH_4^+ \leftrightarrow NH_3(g) + H^+$.

High pH and temperature favors the dissolved ammonia gas form. Ammonia gas has toxic effects at a much lower concentration.

Heavy metal toxicity and toxic organics have been blamed for digester failure, however, they are seldom present in significant concentrations in domestic waste sludges unless large quantities of industrial wastes are discharged to the sewer system.

3.2.2.4 Nutrient Requirements

Domestic waste sludges are typically not deficient in nitrogen and phosphorus, however trace nutrient requirements for anaerobic metabolism are not well defined.

Nitrogen requirements for anaerobic digestion are a small fraction of that required by aerobic processes. This is because of lower biomass production in the anaerobic process. Phosphorus requirements are approximately 15% of the nitrogen requirements (Speece, 1983).

Speece (1983) indicates four elements: iron; cobalt; nickel; and sulphide have been shown to be necessary nutrients for acetate methanogens to convert

Table 3.3 Stimulatory and Inhibitory Concentrations of Toxic Materials in Anaerobic Digestion
(Adapted from McCarty, 1964b)

Material	Stimulatory	Moderate Inhibition	Strong Inhibition
Na (mg/L)	100-200	3,500-5,500	8,000
K (mg/L)	200-400	2,500-4,500	12,000
Ca (mg/L)	100-200	2,500-4,500	8,000
Mg (mg/L)	75-150	1,000-1,500	3,000
NH_3-N (mg/L)	50-200	-	1,500-3,000[1]
Soluble S^{2-} (mg/L)	0.1-10	100	200

[1] Inhibitory at high pH values.

acetate to methane. However, an availability problem of these trace nutrients can potentially occur. Sulphide compounds of iron, cobalt, and nickel exhibit low solubility. These compounds can precipitate out and become unavailable for use by acetate methanogens.

3.3 Anaerobic Digestion of Chemically Coagulated Sludges

3.3.1 Plant Scale Studies

Information in the literature concerning plant scale studies of anaerobic digestibility of chemical sludges have centered on detection of gross inhibition of the digestion process associated with changes in the overall plant operating performance.

Shannon et al. (1974) conducted plant scale studies to assess th potential effects of alum coagulated primary wastewater sludge on anaerobic digestion. Alum addition increased the amount of sludge requiring digestion. Conditions of increased volatile organic acids and decreased alkalinity and pH were observed in the digester soon after initiation of the alum addition to the primary wastewater. Cessation of gas production and failure eventually occurred. Shannon et al. attributed failure to a two-fold increase in volatile solids loading to the digester. Reduced alkalinity in the incoming chemical sludge was also observed.

Emig (1979) monitored digester performance during alum addition to degritted raw wastewater at Fort Sheridon, Illinois. The digester system exhibited significantly decreased total gas production after 30 days of continuous alum addition. Symptoms of volatile acids accumulation occurred. Failure occurred 45 days after the initial operation began. Subsequent jar tests on the incoming chemically precipitated sludge showed reduced alkalinity and depressed pH. Emig felt low pH of the incoming sludge contributed to system failure. Inhibition of the digester's methanogenic bacteria was the probable cause. Information with respect to changes in volatile solids loading to the digester system from alum addition was lacking.

Hall (1980) reviewed digester performance of several water pollution control plants practicing chemical coagulant addition in Ontario. Plants employing alum addition exhibited symptoms described previously. Plants employing addition of iron salts showed no deleterious effects on digestion performance. Hall attributed this to the low chemical dosages of iron salts (8 to 20 mg/L as Fe) to the wastewaters.

The problems encountered during these plant scale studies can be attributed to inadequacies within existing plant operations to adjust to changes in the overall process due to chemical addition. Shannon et al. (1974) demonstrated reduced anaerobic digestion performance occurred from a higher volatile solids loading. Chemical coagulants aid in removal of colloidal material and, when added to the settleable fraction of volatile solids, increase sludge mass and volume to be handled.

Emig (1979) showed sludge pH can change due to alum precipitation. Alum precipitation is most effective at a pH between 5.5 and 6.5. Continuous feed of a chemical sludge at a pH of 5.5 to 6.0 into an operating digester can have inhibitory effects on the methanogenic bacteria.

Stability of the phosphorus precipitate may also be affected by pH (Hall, 1980). Resolubilization of phosphorus during sludge digestion may occur. If the phosphorus content of a chemical sludge is sufficiently high a small fraction of phosphorus may be released and become a soluble nutrient in the digester supernatant. Since the supernatant is generally returned to the head of a treatment plant, phosphorus can accumulate in the process demanding large quantities of additional chemicals.

3.3.2 Laboratory Studies

Laboratory studies have indicated chemical coagulation of domestic wastewater affects digestibility of the resulting sludges. These studies did not experience the problems encountered at plant scale studies previously discussed. Changes in volatile solids loading, pH, and alkalinity in the feed sludge resulting from chemical addition were compensated for in many of the lab scale studies.

Grigoropoulus et al. (1971) reported lab scale digesters fed alum coagulated waste activated sludge showed

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similar gas production and volatile acids concentration to the control digester during semi-continuous operation. An increase in volatile solids loading to the digesters receiving alum precipitated sludge was observed. However, because the digesters receiving higher volatile solids loadings did not experience any increases in gas production, the results suggest a reduction in performance must have occurred for these digesters. Organic loading rates used in the study were generally lower than EPA (1979) recommended design values for standard rate digestion (0.64 to 1.60 Kg VSS/day/m^3). Therefore, it is reasonable to assume decreased digestion performance was not attributed to excesses in volatile solids loading for the digesters receiving alum coagulated sludge.

Malhotra et al. (1971) studied lab scale digestion performance on sludge derived from coagulation of wastewater with steel pickling liquor containing iron. Results were compared to that of gravity settled sludge from the same wastewater for semi-continuous operation. They found that the iron precipitated sludge did not significantly affect performance indicated by:

volume gas of produced/mass of VS destroyed and
% VS destruction.

Gossett et al. (1979) indicates a more appropriate parameter to assess digestion performance for this study would be:

volume of methane produced/mass of VS fed.

Using the data presented by Malhotra et al., Gossett et al.

calculated a 60 to 69% decrease in performance for the iron precipitated sludge based on:

volume of methane produced/mass of VS fed.

Rindt (1973) and Reed (1975) investigated the effects of alum coagulation on digestion performance. Alum precipitated sludge from water treatment and wastewater treatment plant operations were fed to semi-continuously operated lab scale digesters. Both used total gas production rates as a measure of digestion performance.

Rindt showed similar gas production rates for a control digester fed primary wastewater sludge and a test digester. The test digester was fed a mixture of primary wastewater sludge and water treatment plant alum sludge. A more appropriate parameter to assess digestion performance for this study would be:

gas produced (mL/hr)/VS loading (mg/L) .

A 23% decrease in performance for the digester fed the alum sludge mixture can be demonstrated using data presented by Rindt for the 53rd day of operation (based on the rate of gas produced (mL/hr)/VS loading (mg/L)).

Reed (1975) demonstrated gas production rates were an average 15% lower for a test digester (fed a mixture of primary wastewater sludge and water treatment plant alum sludge) than a control digester (fed primary wastewater sludge). The aluminum concentration in the feed sludge mixture was approximately 600 mg/L (as Al). Volatile solid loadings in the digester receiving the sludge mixture were consistently higher than in the control digester indicating greater adverse effect on digestion performance because of presence of aluminum.

Hsu and Pipes (1973) employed batch experiments to investigate the effects of alum sludge from water treatment operations upon anaerobic digestion. Preformed aluminum hydroxide floc was added to a combination of primary and waste activated sludge and fed to test digesters. The control digester received primary and waste activated sludge only. Volatile solid loadings to all batch digesters were equivalent. Total gas production was monitored to assess digestion performance. Hsu and Pipes were able to demonstrate decreased efficiency at high aluminum concentrations in the test digesters. Total gas production decreased 15% at a concentration of 1,549 mg/L as Al. Hsu and Pipes proposed that the decrease in efficiency was due to retardation of the acid forming bacteria from presence of aluminum hydroxide floc. This was attributed to the observation that pH in the digesters containing the preformed aluminum floc remained between 7.0 and 7.6 for the whole digestion period.

Gossett et al. (1978) dosed wastewater samples with various concentrations of alum and ferric chloride. The resulting chemical sludges were fed to semi-continuous lab scale digesters. All feed sludges were initially adjusted to an equivalent volatile solids concentration. Gossett et al. were able to demonstrate the adverse effects of chemical coagulants on anaerobic digestion. Reduced digestion efficiency, demonstrated by decreased total gas production, was evident immediately for digesters fed aluminum and iron precipitated sludges. Gossett et al. concluded the nature of the adverse chemical effect was apparently one of decreasing biodegradability. Examination of alkalinity data revealed significantly reduced alkalinity in the digesters receiving chemical sludges. Decomposition of organic nitrogen was only one half as great in digesters receiving alum sludges compared to the control digester. Reduced alkalinity in the digesters receiving alum sludge was apparently due to a reduced degree of organic nitrogen catabolism. The importance of alkalinity formation from degradation of organic nitrogen compounds was discussed earlier (section 3.2.2.1).

Recent work by Gossett et al. (1979) and Dentel et al. (1982) focused on characterization of the mechanism(s) by which alum and ferric chloride reduce biodegradability of coagulated organics in chemical sludges. Comparisons of digestibility to determine impaired digestion performance were based on:

total gas production/COD fed (mg/L) .

The mechanism causing digestibility decreases was suggested to be some manner of association of substrate with coagulant floc. The mechanism renders a portion of the organics less accessible and/or less reactive to microorganisms or their extracellular enzymes. This effect was proposed to occur during the coagulation process. The enmeshment of colloidal material may form a barrier around organic solids during chemical coagulation. This could effectively interfere with enzmyatic hydrolysis by the acid forming bacteria during digestion.

3.3.3 Development of Research Objectives

The literature has demonstrated the adverse effects of alum and ferric chloride upon anaerobic digestion. However, there exists a lack of information on chemical coagulant concentrations present during digestion which produce these adverse effects.

Plant scale studies typically involve chemical addition at a specific location in the treatment process (i.e. before primary sedimentation, to the aeration basin, etc.). No attempts have been made to determine the chemical coagulant concentration in the feed sludge or present in the digester. Concern has been focused on changes brought about from chemical sludges (i.e. increased sludge volume to be handled and/or reduced hydraulic retention time, effects of low pH in feed sludge).

Laboratory studies involve addition of a chemical coagulant to wastewater samples obtained from a location similar to those discussed above. The coagulated wastewater is usually allowed to settle and the resulting sludge is adjusted for characteristics such as pH, alkalinity, and volatile solids concentration prior to being fed to lab scale digesters. Again, chemical coagulant concentrations present during the period of digestion are unknown. Studies performed by Gossett et al. (1978), Reed (1975), Rindt (1973), and Grigoropoulus et al. (1971) are examples of this.

The objective of this research was to assess the magnitude of effects chemical coagulants have upon anaerobic digestion by monitoring methane production and measuring concentrations of aluminum or iron present during batch digestion of chemically precipitated sludge. More specifically:

1. to determine if a chemical coagulant dose methane response relationship exists in order to establish threshold values for any adverse effects which may be caused by alum or ferric chloride; and
2. to determine any relationship between the experimental results and digester performance at the City of Calgary Bonnybrook Wastewater Treatment Plant where alum addition is practiced to remove phosphorus.

4. EXPERIMENTAL METHODS AND PROCEDURES

The experimental technique used for this study was the Hungate Serum Bottle Technique. The method was adapted from Fedorak and Hrudey (1984). Tests involved observing a response from batch digesting cultures of chemically coagulated activated sludge and anaerobic seed sludge. Percent methane per gram/litre volatile solids loading (%CH_4 per g/L VS) was the response measured to assess the magnitude of effects on methane production. Percent methane is the precent by volume methane produced (or methane concentration) in the headspace of the culture bottle. Volatile solids loading represented the amount of volatile organics in the bottle at the start of the digestion period.

A response from the test cultures was compared to that of the control culture. Deviation of a response from the test culture below the control indicated decreased methane production. The magnitude of the deviation could provide the basis for a chemical coagulant dose methane response relationship.

4.1 Sludge Derivation

All sludge samples were obtained from the City of Edmonton Goldbar Wastewater Treatment Plant or from Bonnybrook Wastewater Treatment Plant, City of Calgary. Activated sludge samples at Goldbar were obtained from sampling ports for the effluent channel of the aeration basin which carried mixed liquor to the final clarifier.

Activated sludge from Bonnybrook was sampled near the surface of the mixed liquor flow in the effluent channel between the aeration basin and the final clarifier, approximately 7 meters downstream from the point where liquid alum was added to the surface. Anaerobic sludge was sampled from ports located at mid-height of the digester units from both treatment plants.

The basic procedure for all the experiments was the same. The following is a step by step procedure for sludge derivation:

1. Activated sludge was distributed into a series of 2,000 mL plastic graduated cylinders;
2. Contents in each cylinder were air mixed for 30 seconds;
3. Alkalinity, in the form of powdered sodium bicarbonate, was immediately added and contents were air mixed for 60 seconds (see Tables 4.1 and 4.2 for quantities);
4. Chemical coagulant (reagent grade alum or ferric chloride) was added in various concentrations to the cylinders and the contents were air mixed for 120 seconds (see Tables 4.1 and 4.2 for quantities);
5. Sodium hydroxide (1 Normal solution) was added to the cylinders to adjust the pH within 6.5 to 7.5 and the contents were air mixed for 30 seconds (see Tables 4.1 and 4.2 for quantities);
6. Contents in the cylinders were allowed to settle

Table 4.1 TREATMENT CONDITIONS FOR ALUM PRECIPITATED SLUDGE DERIVATION

			EXPERIMENTAL RUNS						
ALUM (mg) (1)(2)	ALKALINITY (mg) (2)		R1	R2	R3	R4	R5	R8	(3)
0	*0*	CONTROL	X	X	X	X	X	X	0
100	*50*	I	X	X					0
200	*100*	II	X	X	X	X	X	X	0
400	*200*	III	X	X		X	X		1.3
600	*300*	IV	X	X	X	X	X	X	2.0
800	*450*	V	X	X		X	X		2.4
1,000	*600*	VI	X		X			X	3.4
1,200	*750*	VII	X			X	X		4.3
1,600	*1,000*	VIII	X		X	X	X	X	7.0
2,000	*1,200*	IX	X			X	X		8.3

(1) Reagent grade alum, formula weight 666.42 .
(2) Amount added to 2,000 mL of activated sludge.
(3) mL of 1 N NaOH added to 2,000 mL of activated sludge.

NOTE: X indicates treatment conditions used for experimental runs.

Table 4.2 TREATMENT CONDITIONS FOR FERRIC CHLORIDE PRECIPITATED SLUDGE DERIVATION

FERRIC CHLORIDE (mg) (1)(2)	ALKALINITY (mg) (1)		EXPERIMENTAL RUNS			
			R1	R2	R3	(3)
0	0	CONTROL	X	X	X	0
100	100	I	X			0
200	200	II	X	X	X	1.0
400	400	III	X	X		2.0
600	700	IV	X	X	X	3.0
1,000	1,200	V	X	X	X	6.0
1,500	1,600	VI	X	X	X	9.0
2,000	2,000	VII	X			11.5
2,500	2,500	VIII	X			14.0
3,000	3,000	IX	X			16.0

(1) Anhydrous ferric chloride, formula weight 162.22 .
(2) Amount added to 2,000 mL of activated sludge.
(3) mL of 1 N NaOH added to 2,000 mL of activated sludge.

NOTE: X indicates treatment conditions used for experimental runs.

under quiescent conditions for 90 minutes (activated sludge used for the control was air mixed for a total of 4 minutes and allowed to settle);

7. A vacuum pump was used to draw off the supernatant (an equivalent amount of supernatant was removed from each cylinder in Alum Experiment R6. The remaining contents were gently remixed. This attempted to equalize the volume of settled sludge in each cylinder); and
8. A representative sample of settled sludge from each cylinder (150 to 175 mL volume) was transferred to a 250 mL Erlenmeyer flask and rubbered stoppered for approximately 45 minutes prior to being added to the serum bottles.

Tables 4.1 and 4.2 show the amounts of chemical coagulant (alum and ferric chloride) and sodium bicarbonate added to 2 litres of activated sludge.

A sufficient volume of settled sludge from each cylinder was later used for wastewater characterization tests.

4.2 Experimental Program

4.2.1 Alum Experiments

The following is a discussion of each experiment performed.

Alum R1 Initial experiment to determine if an aluminum dose methane response relationship exists.

Alum R2 The purpose was to determine if the results are reproducible within an experiment for any treatment condition tested. Two independent samples of alum precipitated settled sludge were derived for each treatment condition and used for the batch culture assays.

Alum R3 The purpose was to investigate the effects of alum on acetate utilizing methanogenic bacteria. Each sample of aluminum precipitated sludge was tested with an acetate supplement present and without it. Acetate is a volatile organic acid that serves as a substrate for acetate methanogens. In this way, the acetate methanogens are not dependent upon the non-methanogens to produce acetate. Acetate supplement (75 mg/mL) was added as a substrate for the acetate methanogens (17.29 g $CH_3COONa \cdot 3H_2O$ dissolved in 100 mL of distilled water and adjusted to pH 7.0 with NaOH). A like volume of distilled water was used for each treatment condition in place of the acetate supplement for comparison.

Alum R4 The purpose was to determine if the results of experiment R1 are reproducible.

Alum R5 The purpose was to determine any relationship between the previous experiments and digester performance at the City of Calgary Bonnybrook Wastewater Treatment Plant. The plant employs alum addition prior to the final clarifier. An equivalent alum dosage of

70 mg ($Al_2(SO_4)_3 \cdot 14H_2O$) is added per L of wastewater treated in the form of a liquid (48.5% by weight dry $Al_2(SO_4)_3 \cdot 14H_2O$ in water). Activated sludge samples from Bonnybrook (already containing alum from plant operations) were spiked with various amounts of reagent grade alum. Anaerobic sludge from Bonnybrook was used as seed. The resulting test conditions were compared to the control (containing only alum from plant operations) to try to demonstrate an aluminum dose methane response relationship.

Alum R6 The purpose was to investigate the effects of alum on alkalinity production under batch operations. Each sample of aluminum precipitated sludge derived was tested in the presence of normal alkalinity and an alkalinity supplement. The alkalinity supplement was provided by addition of a 30 g/L solution of sodium bicarbonate. Normal alkalinity represented the amount of alkalinity present in the serum bottles (alkalinity conditions at which all previous experiments were tested). A like volume of distilled water was used for each treatment condition (instead of the sodium bicarbonate supplement) for normal alkalinity conditions. Extra serum bottles were set up for each treatment condition. One bottle from each treatment condition was opened every 14 days during incubation and analyzed for alkalinity.

4.2.2 Ferric Chloride Experiments

The following is a discussion of the experimental runs performed.

$FeCl_3$ R1 Initial experiment to determine if an iron dose methane response relationship exists.

$FeCl_3$ R2 The purpose was to determine if the results are reproducible within an experiment for any treatment condition tested. Two independent samples of iron precipitated settled sludge were derived for each treatment condition and used for the batch culture assays.

$FeCl_3$ R3 The purpose was to investigate the effects of ferric chloride on acetate utilizing methanogenic bacteria. Each sample of iron precipitated sludge derived was tested with acetate present and without it. An acetate supplement (75 mg/mL) was added as a substrate for acetate methanogens. A like volume of distilled water was used for each treatment condition in place of the acetate supplement for comparison.

4.3 Anaerobic Bioassay Technique

The batch culture assays were performed in Type 1 borosilicate glass, 150 mL serum bottles (Wheaton Scientific, 158 mL actual volume). Triplicate cultures were set up for each treatment condition. During each experimental setup the serum bottles were continuously gassed with a mixture of O_2 free 30% CO_2 and 70% N_2.

Anaerobic sludge, used as seed, was placed in an open 1,000 mL Erlenmeyer flask on a magnetic stirring plate. The headspace above the liquid level of the sludge was continuously gassed with the O_2 free $CO_2:N_2$ mixture.

Chemically precipitated settled sludge in the 250 mL Erlenmeyer flask was sparged with N_2 gas for a period of 15 minutes prior to being added to the serum bottles. The sparging attempted to remove any oxygen trapped in the sludge. The headspace above the liquid level of the settled sludge was continuously gassed with N_2.

Separate pipettes were used for transferring aliquots of each sample of settled sludge and anaerobic sludge to the serum bottles. Transfer of sludge was accomplished in the following manner. A piece of 600 mm long removable latex rubber tubing was attached to the top of the pipette. Suction was provided by placing the other end of the tubing in the mouth. Prior to drawing sludge into the pipette, the volume in the pipette and the tubing were filled with gas (used to flush the headspace of the sludge) by suction. This procedure was to minimize oxygen contamination. An aliquot of sludge was then drawn into the pipette and transferred to the serum bottle. This procedure was repeated for each transfer of aliquot.

The transfer of other constituents to serum bottles were performed by regular pipetting techniques.

The procedure followed for Alum Experiments R1, R2, R4, and R5; and Ferric Chloride Experiments R1 and R2 was as follows:

1. Add 1 mL resazurin (a redox indicator that turns from pink to colorless when it is reduced);
2. Add 19 mL aliquot of anaerobic sludge;
3. Add 30 mL aliquot of chemically precipitated settled sludge; and
4. Seal serum bottles with butyl rubber stoppers and aluminum seal.

The liquid volume in the serum bottles (50 mL) consisted of:

- 1 mL redox indicator (2% by volume);
- 19 mL anaerobic seed sludge (38% by volume); and
- 30 mL settled sludge (60% by volume).

The procedure followed for Alum Experiment R3 and Ferric Chloride Experiment R3 was as follows:

1. Add 1 mL resazurin;
2. Add 0.5 mL distilled water or 75 mg/mL acetate supplement;
3. Add 19 mL aliquot of anaerobic sludge;
4. Add 30 mL aliquot of settled sludge; and
5. Seal the serum bottles.

The liquid volume in the serum bottles (50.5 mL) consisted of:

- 0.5 mL distilled water or acetate supplement (1% by volume);
- 1 mL redox indicator (2% by volume);
- 19 mL anaerobic seed sludge (37.6% by volume); and
- 30 mL settled sludge (59.4% by volume).

The procedure followed for Alum Experiment R6 was as follows:

1. Add 1 mL resazurin;
2. Add 5 mL of either distilled water or 30 g/L supplement of sodium bicarbonate (alkalinity);
3. Add 19 mL aliquot of anaerobic seed sludge;
4. Add 25 mL aliquot of settled sludge; and
5. Seal the serum bottles.

The liquid volume in the serum bottles (50 mL) consisted of:

- 1 mL redox indicator (2% by volume);
- 5 mL distilled water or alkalinity supplement (10% by volume);
- 19 mL anaerobic sludge (38% by volume); and
- 25 mL settled sludge (50% by volume).

The serum bottles were placed in an incubator at 37 °C for 55 to 60 days. During the initial stages of incubation samples of the headspace gas were removed every 2 to 3 days (less frequently later) and analyzed for methane by gas chromatography.

Calculated sodium concentrations in the serum bottles from the addition of sodium bicarbonate (alkalinity) and sodium hydroxide (pH adjustment) during sludge preparation were below moderate inhibitory levels indicated in Table 3.3 (3,500 to 5,500 mg/L).

4.4 Analytical Methods

Surplus samples of the chemically precipitated settled sludge and anaerobic sludge from all experiments were added to 500 mL borosilicate glass containers in the same percent volume proportions as discussed in section 4.3. Distilled water was used in place of resazurin and acetate. The 500 mL samples contained the same components of interest as samples in the serum bottles. These sludge samples were preserved according to Standard Methods (APHA 1980, Section 105) and used for alkalinity, residue, and metal analyses (Al and Fe).

4.4.1 Alkalinity

All sludge samples were centrifuged for 30 minutes at 3000 rpm with a Sorvall RC-5B Refrigerated Superspeed Centrifuge (Du Pont Instruments). Alkalinity determinations were made on the decanted supernatant at room temperature.

Alkalinity was determined in accordance with Standard Methods (APHA 1980, Section 403). Ten mL sample volumes were titrated with 0.02 N standardized sulphuric acid solution. A mixed bromocresol green methyl red solution was used to indicate a color change from blue to light pink ($pH \approx 4.6$). Triplicate determinations were made for all samples, average values were recorded.

4.4.2 Residue

Total solids were determined from the method outlined by Jenkins et al. (1980). Sludge (25 mL sample) was placed in a prepared aluminum evaporating dish, allowed to evaporate to dryness on a steam bath, and oven dried for 1 hour at 103 °C. Volatile solids were determined by ignition for 30 minutes at 550 °C of residue from total solids analyses. All determinations were made in triplicate, average values were recorded.

An attempt was made to correct for any source of contribution to volatile solids from the alkalinity reactions with alum or ferric chloride during the coagulation process. Volatile solids were also determined for all treatment conditions listed in Tables 4.1 and 4.2 using tap water blanks (50 mL samples). Volatile solids determined from these analyses represented the volatile solids contribution by alum and ferric chloride and these were subtracted from volatile solids determined from analyses of each wastewater treatment condition with the sludge samples. The resulting value more accurately represented the amount of wastewater volatile organics present in the serum bottles at the beginning of the digestion period.

4.4.3 Methane Analysis

Methane measurements were determined by gas chromatography (GC) methods. A Hewlett-Packard (GC) (Model

5730A) equipped with a flame ionization detector was fitted with a 0.90 m 1 mm ID glass column packed with 1% SP-1240A DA on 100/120 Supelcoport (Supelco). Nitrogen was used as a carrier gas at 30 mL/min with hydrogen and air flows of 30 mL/min and 240 mL/min respectively. The oven, injector, and detector temperatures were 25, 25, and 100 °C respectively. A Hewlett-Packard System Integrator (Model 3385A) was used in the determination of percent methane.

A series of standards (known concentrations of methane) were analyzed on the GC prior to each methane analysis of the batch cultures. Methane standards were prepared in 158 mL volume serum bottles. Percent methane in the standards were determined from the following formula:

$\%CH_4 = (a/(a+158))\cdot 100$; where

a = volume (mL) of purified methane added to the bottle.

A relationship between the percent by volume methane in the standards and a corresponding value indicated by the integrator was subject to linear regression. The resulting regression equation was applied to integrator values obtained from analysis of the batch culture assays to yield $\%CH_4$.

Corrections were made for the presence of water vapor in the samples taken for GC analysis. Observed methane concentrations were divided by a correction factor determined at ambient temperature at the time of analysis. The correction factor (CF) was determined from the following formula:

$$CF = 1 - ((a/b)\cdot c/1{,}000)$$; where

a = amount of H_2O in saturated air at ambient temperature at time of analysis (g H_2O/m^3 or g H_2O/1000 litres) obtained from List (1949);

b = gram molecular weight of H_2O (= 18 g/mole); and

c = volume occupied per mole of gas at standard temperature and pressure (STP) (= 22.4 L/mole).

Methane sampling and removal during incubation was performed with plastic syringes (0.5 mL) equipped with 28 gauge - 12 mm long needles. The sampling syringe was flushed with nitrogen gas prior to each individual measurement. All syringe measurements were taken at room temperature.

4.4.4 Sludge Digestion For Metal Analyses

Sludge samples were steam digested under pressure to solubilize the metals prior to determination of Al and Fe. The rapid digestion method outlined by Nielsen and Hrudey (1984) was followed.

Sludge samples were acidified (~1% v/v) with concentrated HNO_3 and then 20 mL aliquots were transferred to 50 mL Pyrex test tubes. The test tubes were covered, placed in a template stand in a pressure cooker, and steam digested for 1 hour at 210 KPa (2 atm). Iron sludge samples were covered with aluminum foil and aluminum sludge samples were covered with polyester wrapping (Look Bags, Reckitt and Coleman Canada Inc, Lachine, Que.) and held in place by aluminum foil. Blanks (distilled water) were steam digested to account for metal contamination from the pressure cooker. The digested supernatant was decanted, filtered, and analyzed.

Iron sludge samples were steam digested in an aluminum Model 411 domestic pressure cooker of 11 L capacity (Presto, Scarborough, Ontario). Aluminum sludge samples were steam digested in a stainless steel Model 18/10 domestic pressure cooker of 5 L capacity (Lagostina, Italy).

4.4.5 Metal Analyses

Metals (Al and Fe) were determined by flame atomic absorption spectroscopy using a Model 5000 spectrophotometer (Perkin-Elmer, Norwalk, CT). Metals were determined in accordance with Standard Methods (APHA 1980, Section 303C for Al and Section 303A for Fe). Operating conditions for the flame atomic absorption metal determinations are listed in Table 4.3.

Stock standards for Al and Fe (Fisher Scientific) were used to develop calibration curves with respect to metal concentration and absorbance value. All standards and samples were diluted so that their absorbance values fell on the linear part of the respective calibration curves. Blanks and standards were analyzed after every fifth sample to correct for instrumental drift and changes in flame operating conditions. Triplicate readings of all samples and standards were made, average values were recorded.

Table 4.3 **OPERATING CONDITIONS FOR FLAME ATOMIC ABSORPTION METAL DETERMINATION**

CONDITIONS	**METAL**	
	Fe	**Al**
Wavelength	*309.3 nm*	*302.1 nm*
Slit width	*0.7 nm*	*0.2 nm*
Light source	Hollow cathode lamp	Hollow cathode lamp
Flame type	Nitrous oxide-acetylene reducing flame	Air-acetylene oxidizing flame

4.4.6 Statistical Methods

Methane production per volatile solids data from the bioassays were analyzed using the method of Dunnett (1955) to determine which treatment conditions produced mean responses (%CH_4 per g/L VS) significantly less than the mean control response ($P < 0.05$). Triplicate cultures for each test condition and control were set up. A mean of the three cultures was taken as the response for each test condition and compared to the mean for the control using the Dunnett method.

5. EXPERIMENTAL RESULTS

5.1 Wastewater Characteristics

Tables 5.1 to 5.9 show the wastewater characteristics of interest for each treatment condition in the bioassays. Alkalinity information for Alum Experiment R6 is presented in Figures 5.1 and 5.2. Figures 5.1 and 5.2 were derived from data presented in Appendix I.

5.2 Bioassays

Trends in methane production, on a " per mass of volatile solids loading" basis, for the bioassays are presented in Figures 5.3 to 5.14. Some treatment conditions from each bioassay have been omitted from the figures for clarity. Results for all the bioassays are tabulated in Appendix II. The tables in Appendix II indicate values for the lower end of a non-significant range for methane production per volatile solids loading (determined from the method of Dunnett, 1955). Significant adverse effects in methane production (compared to the control) are indicated when methane production per volatile solids loading for any treatment condition is lower than this value ($P < 0.05$).

5.3 Methane Generation

Linear chemical coagulant dose methane response relationships for the bioassays are presented in Figures 5.15 to 5.25. Percent methane per g/L VS loading values at the end of the 3rd, 6th, and 8th week of incubation (approximate times) were used to calculate an average value for the methane generation of each treatment condition. The average values are plotted in the methane generation figures (Figures 5.15 to 5.25). Methane generation and $\%CH_4$ per g/L VS loading for the three incubation times chosen are shown in Appendix III.

The linear relationships demonstrated in the methane generation figures should not be used as empirical relationships for prediction of methane response. Significance of the slope, however, may give some indication as to whether or not a functional relationship exists between chemical concentration and methane production.

5.4 Significance Tests On Chemical Coagulant Concentration Methane Response Relationship

Significance tests were performed on the methane generation data presented in section 5.3 to determine if a functional coagulant dose methane response relationship exists. The F test using the analysis of variance (ANOVA) approach (Neter and Wasserman, 1974) was used to check whether the slopes of the methane generation relationships were significantly different from 0 ($P < 0.05$). Calculations are presented in Appendix IV. Results of the significance tests are summarized for the alum experiments (Table 5.10) and ferric chloride experiments (Table 5.11).

5.5 Chemical Coagulant Effects Upon Acetate Methanogenic Bacteria

Tables 5.12 and 5.13 compare methane generation for aluminum and iron precipitated sludge with and without acetate supplementation. The tables were prepared from data presented in Appendix III.

Table 5.1 Alum Experiment R1

WASTEWATER CHARACTERISTICS

	ALKALINITY (mg/L)[1]	TOTAL SOLIDS (mg/L)	VOLATILE SOLIDS (g/L)	Al (mg/L)
CONTROL	*910*	*4,000*	*2.71* *(2.76)*[2]	*22*
I	*910*	*3,660*	*2.39* *(2.44)*	*24*
II	*940*	*3,920*	*2.57* *(2.63)*	*39*
III	*970*	*4,030*	*2.58* *(2.64)*	*52*
IV	*1,000*	*3,790*	*2.35* *(2.42)*	*62*
V	*1,010*	*3,770*	*2.28* *(2.36)*	*69*
VI	*1,030*	*3,680*	*2.13* *(2.23)*	*78*
VII	*1,060*	*3,760*	*2.11* *(2.21)*	*87*
VIII	*1,080*	*3,660*	*1.95* *(2.07)*	*94*
IX	*1,120*	*3,800*	*1.90* *(2.02)*	*110*

[1] As $CaCO_3$.

[2] Volatile solids uncorrected for contributions by alkalinity reactions (see section 4.4.2).

NOTE: Values represent amount of constituents present at beginning of incubation.

Table 5.2 Ferric Chloride Experiment R1

WASTEWATER CHARACTERISTICS

	ALKALINITY (mg/L)[1]	TOTAL SOLIDS (mg/L)	VOLATILE SOLIDS (g/L)	Fe (mg/L)
CONTROL	890	4,170	2.85 (2.91)[2]	9
I	900	3,770	2.43 (2.51)	31
II	920	3,830	2.45 (2.54)	56
III	950	4,060	2.45 (2.56)	120
IV	970	4,000	2.30 (2.41)	153
V	1,010	3,880	1.98 (2.12)	211
VI	1,060	3,740	1.66 (1.82)	246
VII	1,130	4,240	1.77 (1.95)	354
VIII	1,210	5,420	2.18 (2.39)	622
IX	1,230	5,820	2.17 (2.40)	770

[1] As $CaCO_3$.

[2] Volatile solids uncorrected for contributions by alkalinity reactions (see section 4.4.2).

NOTE: Values represent amount of constituents present at beginning of incubation.

Table 5.3 Alum Experiment R2

WASTEWATER CHARACTERISTICS

	ALKALINITY (mg/L)[1]	TOTAL SOLIDS (mg/L)	VOLATILE SOLIDS (g/L)	Al (mg/L)
CONTROL	*1,070*	*3,460*	*2.29* *(2.34)*[2]	*20*
O	*1,050*	*3,470*	*2.34* *(2.39)*	*19*
I (a)	*1,070*	*4,100*	*2.81* *(2.86)*	*30*
I (b)	*1,080*	*4,130*	*2.83* *(2.88)*	*30*
II (a)	*1,100*	*4,820*	*3.37* *(3.43)*	*43*
II (b)	*1,100*	*4,820*	*3.30* *(3.36)*	*44*
III (a)	*1,110*	*5,170*	*3.52* *(3.58)*	*71*
III (b)	*1,120*	*5,260*	*3.59* *(3.65)*	*78*
IV	*1,140*	*4,980*	*3.26* *(3.33)*	*87*
V	*1,170*	*5,270*	*3.43* *(3.51)*	*110*

[1] As $CaCO_3$.

[2] Volatile solids uncorrected for contributions by alkalinity reactions (see section 4.4.2).

NOTE: Values represent amount of constituents present at beginning of incubation.

Table 5.4 Ferric Chloride Experiment R2

WASTEWATER CHARACTERISTICS

	ALKALINITY (mg/L)[1]	TOTAL SOLIDS (mg/L)	VOLATILE SOLIDS (g/L)	Fe (mg/L)
CONTROL	1,070	2,980	1.91 (1.97)[2]	7
O	1,070	2,960	1.86 (1.92)	7
II (a)	1,110	3,430	2.14 (2.23)	36
II (b)	1,100	3,250	2.00 (2.09)	32
III (a)	1,130	3,600	2.17 (2.28)	69
III (b)	1,120	3,740	2.27 (2.38)	75
IV (a)	1,130	3,710	2.13 (2.24)	103
IV (b)	1,150	3,660	2.09 (2.20)	100
V (a)	1,210	3,890	2.02 (2.16)	161
V (b)	1,220	3,850	2.00 (2.14)	157
VI (a)	1,270	4,300	2.07 (2.23)	250
VI (b)	1,270	4,240	2.01 (2.17)	244

[1] As $CaCO_3$.

[2] Volatile solids uncorrected for contributions by alkalinity reactions (see section 4.4.2).

NOTE: Values represent amount of constituents present at beginning of incubation.

Table 5.5 Alum Experiment R3

WASTEWATER CHARACTERISTICS

	ALKALINITY (mg/L)[1]	TOTAL SOLIDS (mg/L)	VOLATILE SOLIDS (g/L)	Al (mg/L)
CONTROL	1,090	3,100	2.11 (2.16)[2]	20
II	1,140	3,840	2.66 (2.72)	33
IV	1,180	4,050	2.65 (2.72)	60
VI	1,230	4,080	2.60 (2.70)	85
VIII	1,280	4,120	2.44 (2.56)	108

[1] As $CaCO_3$.

[2] Volatile solids uncorrected for contributions by alkalinity reactions (see section 4.4.2).

NOTE: Values represent amount of constituents present at beginning of incubation.

Table 5.6 Ferric Chloride Experiment R3

WASTEWATER CHARACTERISTICS

	ALKALINITY (mg/L)[1]	TOTAL SOLIDS (mg/L)	VOLATILE SOLIDS (g/L)	Fe (mg/L)
CONTROL	*1,060*	*3,120*	*2.01* *(2.07)*[2]	*8*
II	*1,100*	*3,220*	*1.97* *(2.06)*	*36*
IV	*1,160*	*3,490*	*1.95* *(2.06)*	*98*
V	*1,230*	*3,920*	*2.03* *(2.17)*	*176*
VI	*1,290*	*4,920*	*2.43* *(2.59)*	*364*

[1] As $CaCO_3$.

[2] Volatile solids uncorrected for contributions by alkalinity reactions (see section 4.4.2).

NOTE: Values represent amount of constituents present at beginning of incubation.

Table 5.7 Alum Experiment R4

WASTEWATER CHARACTERISTICS

	ALKALINITY (mg/L)[1]	TOTAL SOLIDS (mg/L)	VOLATILE SOLIDS (g/L)	Al (mg/L)
CONTROL	1,050	2,720	1.73 (1.78)[2]	16
II	1,090	3,430	2.23 (2.29)	28
III	1,120	3,480	2.18 (2.24)	39
IV	1,150	3,610	2.22 (2.29)	56
V	1,170	3,750	2.25 (2.32)	67
VII	1,200	3,950	2.27 (2.37)	90
VIII	1,230	4,130	2.27 (2.39)	118
IX	1,270	4,340	2.29 (2.41)	144

[1] As $CaCO_3$.

[2] Volatile solids uncorrected for contributions by alkalinity reactions (see section 4.4.2).

NOTE: Values represent amount of constituents present at beginning of incubation.

Table 5.8 Alum Experiment R5

WASTEWATER CHARACTERISTICS

	ALKALINITY (mg/L)[1]	TOTAL SOLIDS (mg/L)	VOLATILE SOLIDS (g/L)	Al (mg/L)
CONTROL	*1,290*	*14,240*	*9.20* *(9.25)*[2]	*647*
II	*1,310*	*15,300*	*9.92* *(9.98)*	*733*
III	*1,330*	*15,090*	*9.72* *(9.78)*	*733*
IV	*1,360*	*15,230*	*9.73* *(9.80)*	*739*
V	*1,380*	*15,310*	*9.77* *(9.85)*	*766*
VII	*1,430*	*15,540*	*9.79* *(9.89)*	*772*
VIII	*1,450*	*15,300*	*9.56* *(9.68)*	*778*
IX	*1,530*	*15,950*	*9.87* *(9.99)*	*806*

[1] As $CaCO_3$.

[2] Volatile solids uncorrected for contributions by alkalinity reactions (see section 4.4.2).

NOTE: Values represent amount of constituents present at beginning of incubation.

Table 5.9 Alum Experiment R6

WASTEWATER CHARACTERISTICS

	TOTAL SOLIDS (mg/L)	VOLATILE SOLIDS (g/L)	Al (mg/L)
CONTROL	2,810	1.87 (1.92)[1]	10
II	2,920	1.88 (1.94)	21
IV	3,140	1.93 (2.00)	47
VI	3,310	1.94 (2.04)	80
VIII	3,570	1.96 (2.08)	110

[1] Volatile solids uncorrected for contributions by alkalinity reactions (see section 4.4.2).

NOTE: Values represent amount of constituents present at beginning of incubation.

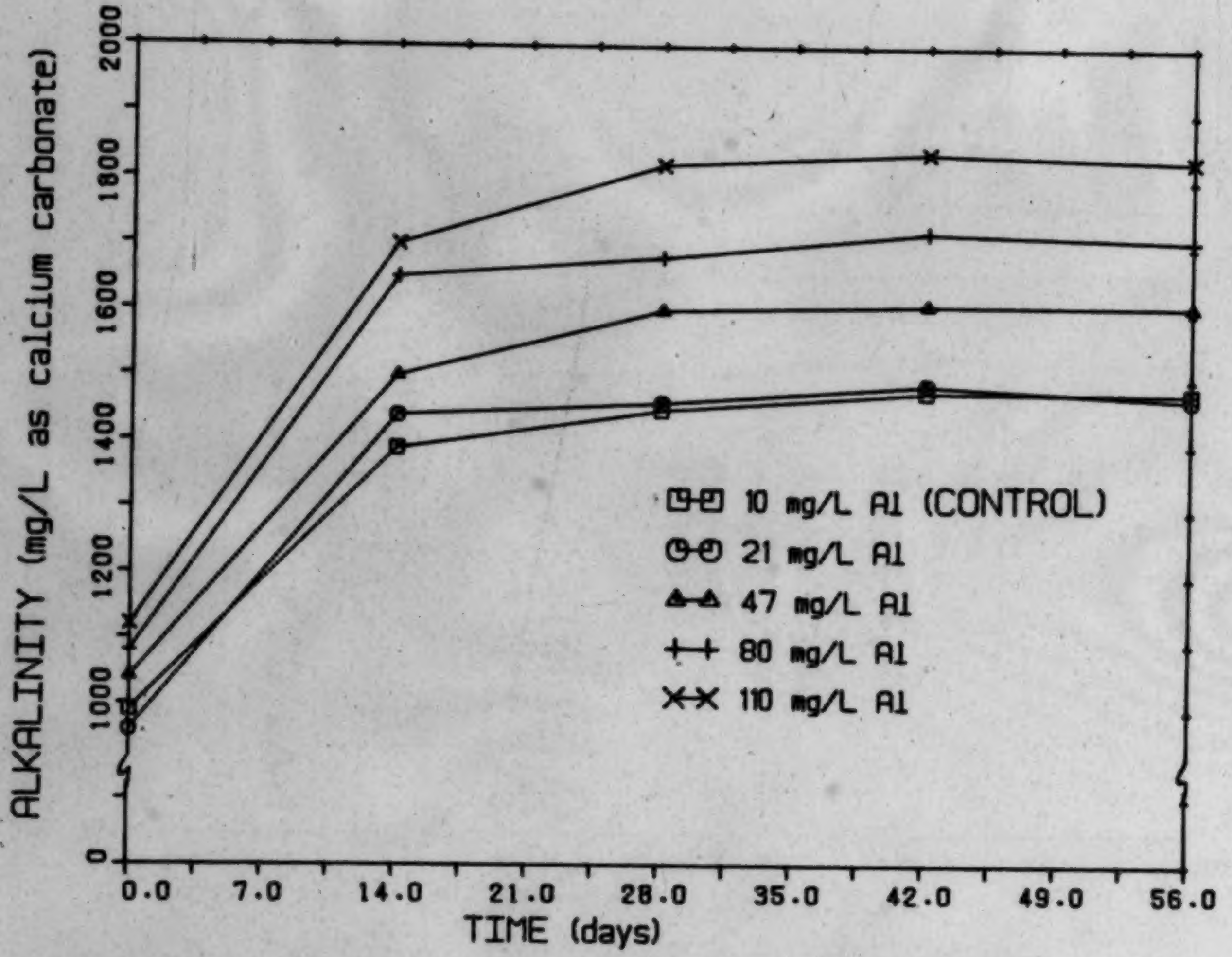

Figure 5.1 Alum Experiment R6 Alkalinity Data

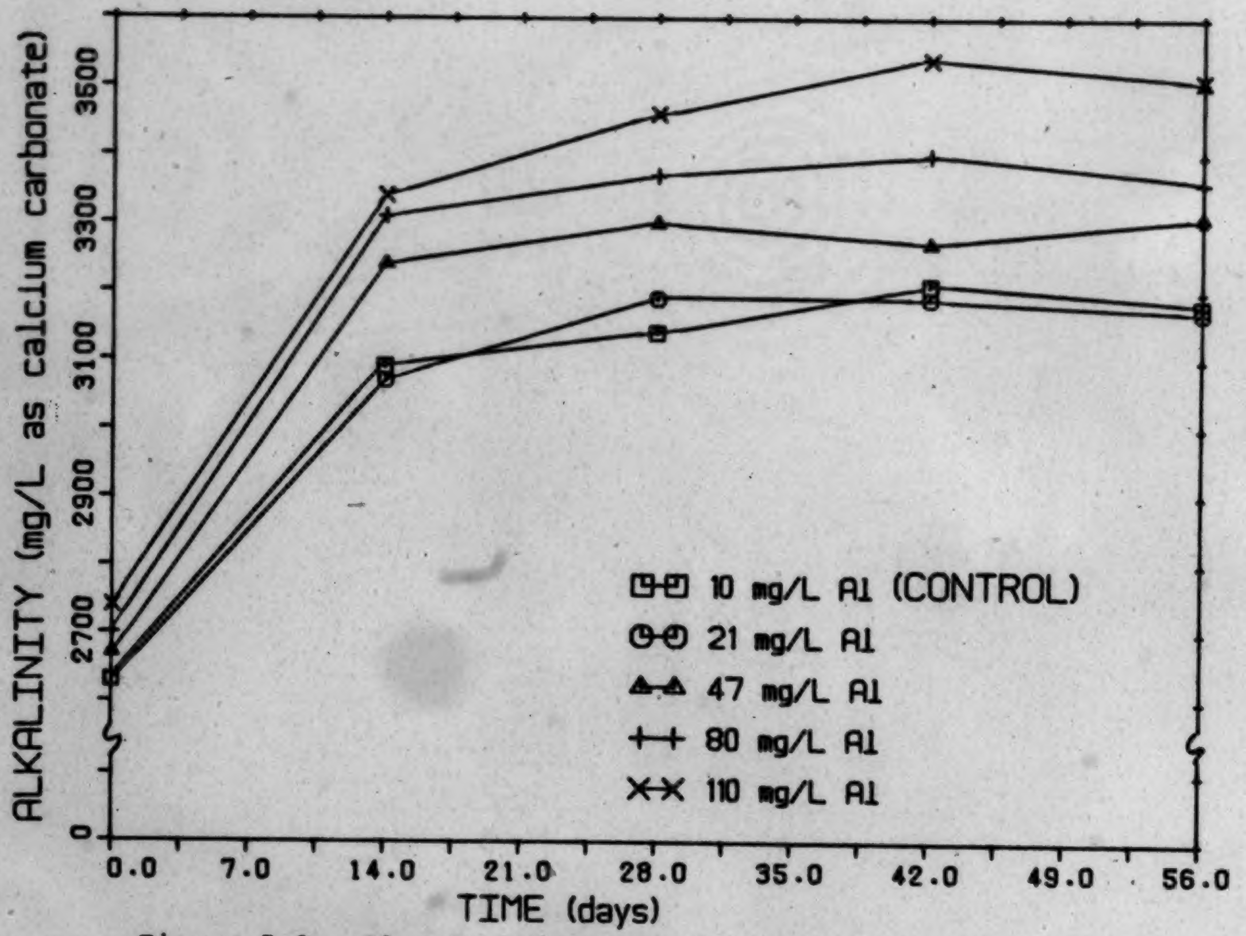

Figure 5.2 Alum Experiment R6 (With Alklalinity Supplement)
Alkalinity Data

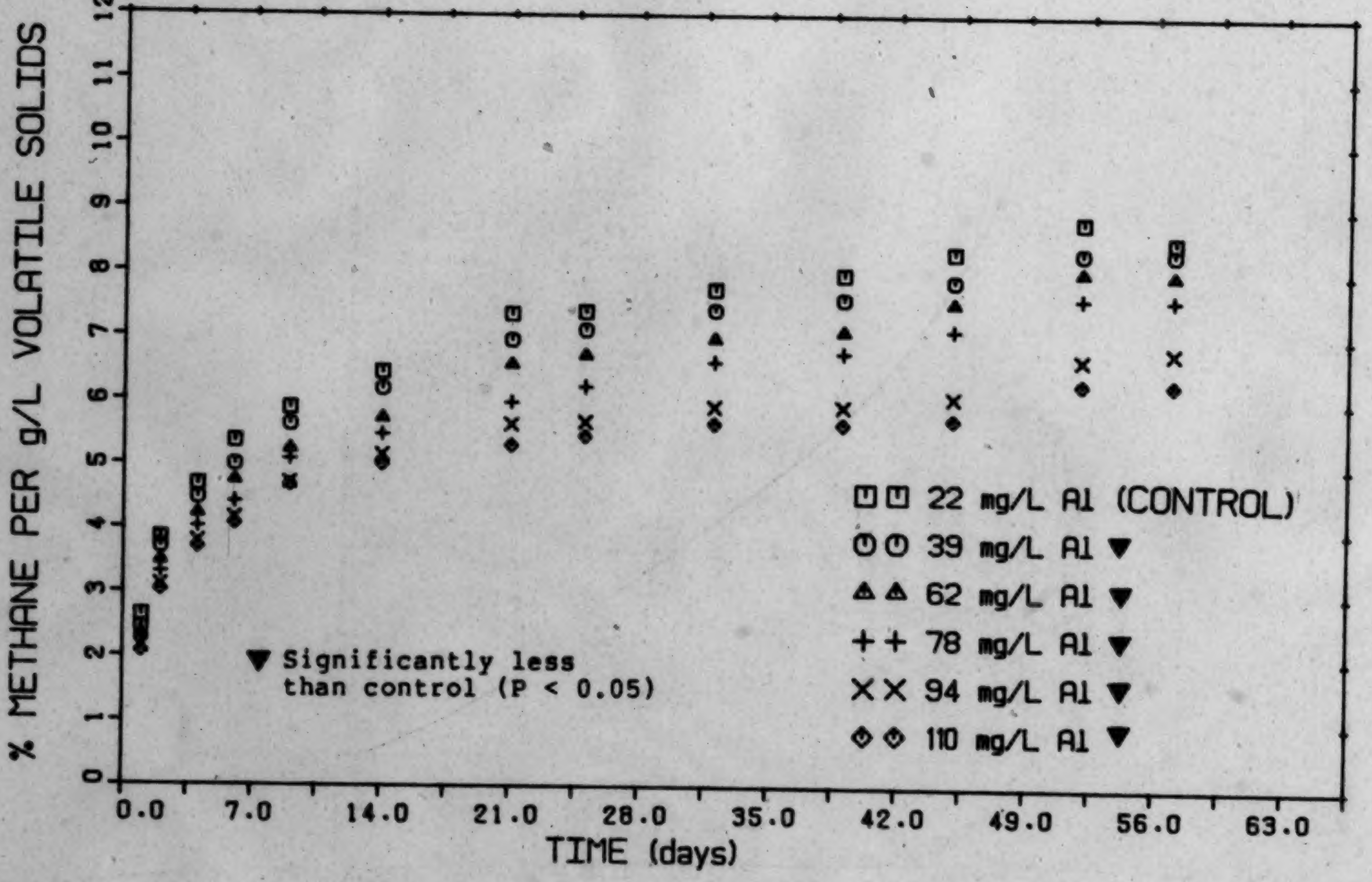

Figure 5.3 Bioassay Test - Alum Experiment R1

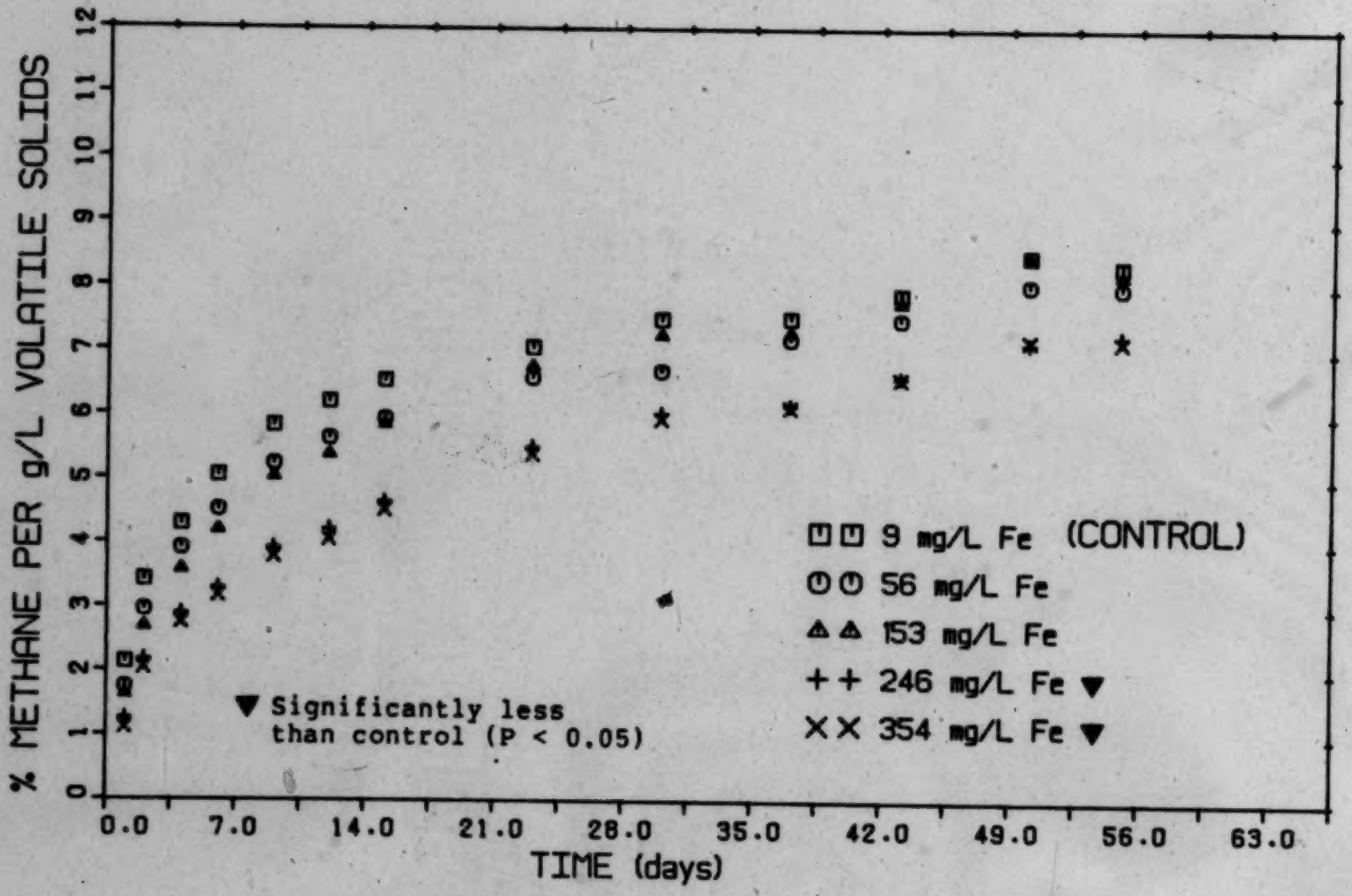

Figure 5.4 Bioassay Test - Ferric Chloride Experiment R1

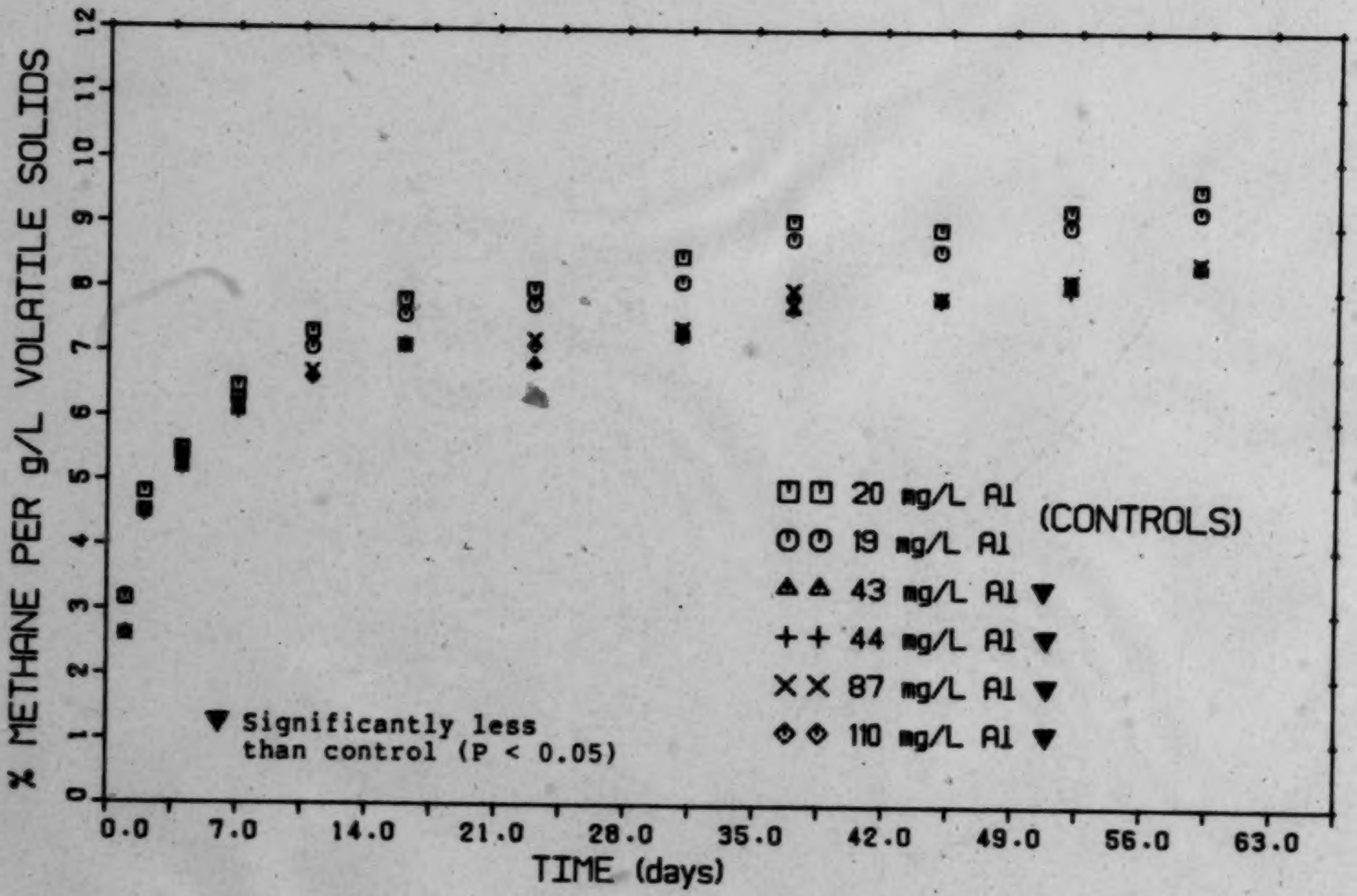

Figure 5.5 Bioassay Test - Alum Experiment R2

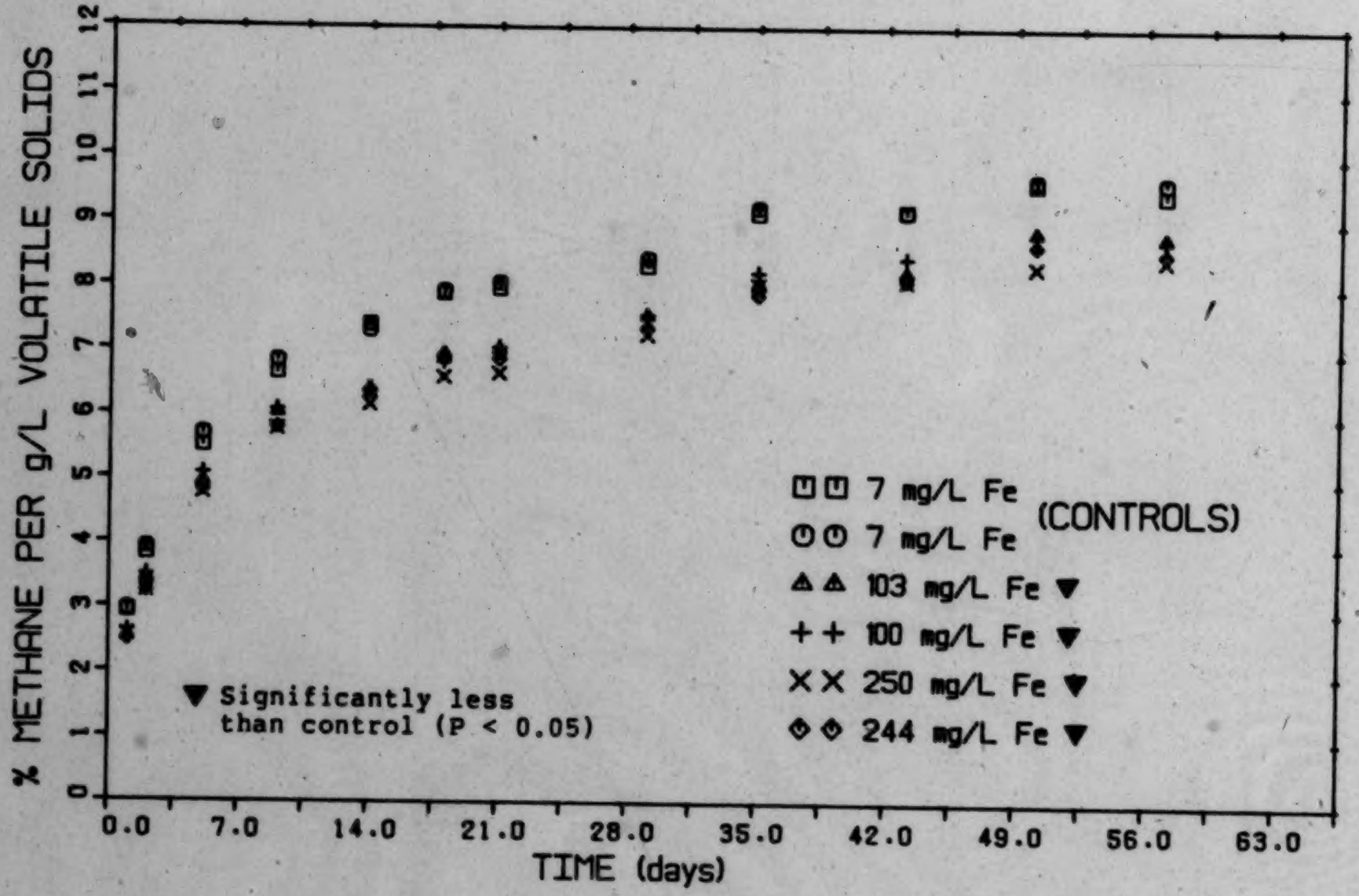

Figure 5.6 Bioassay Test - Ferric Chloride Experiment R2

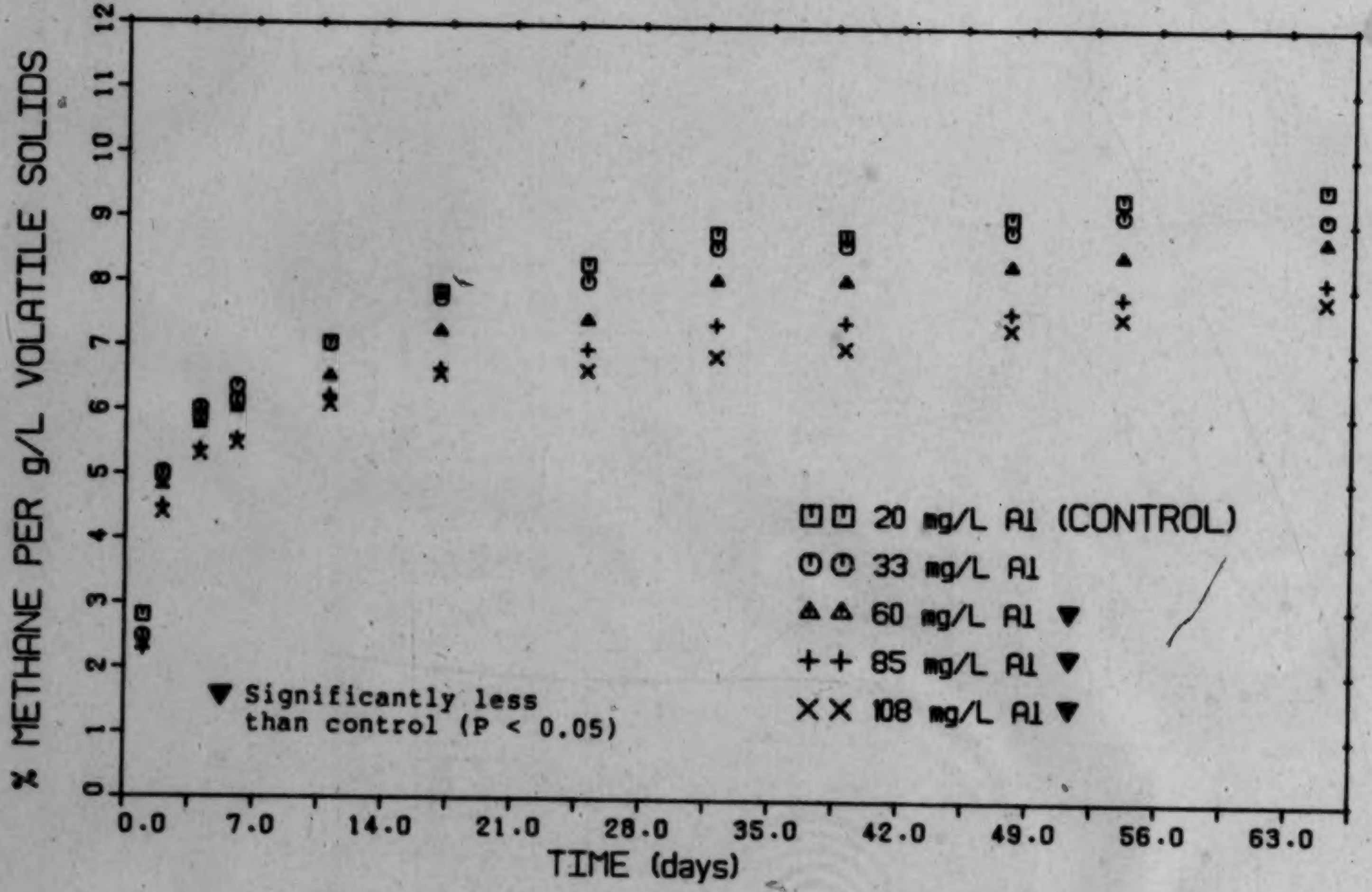

Figure 5.7 Bioassay Test - Alum Experiment R3

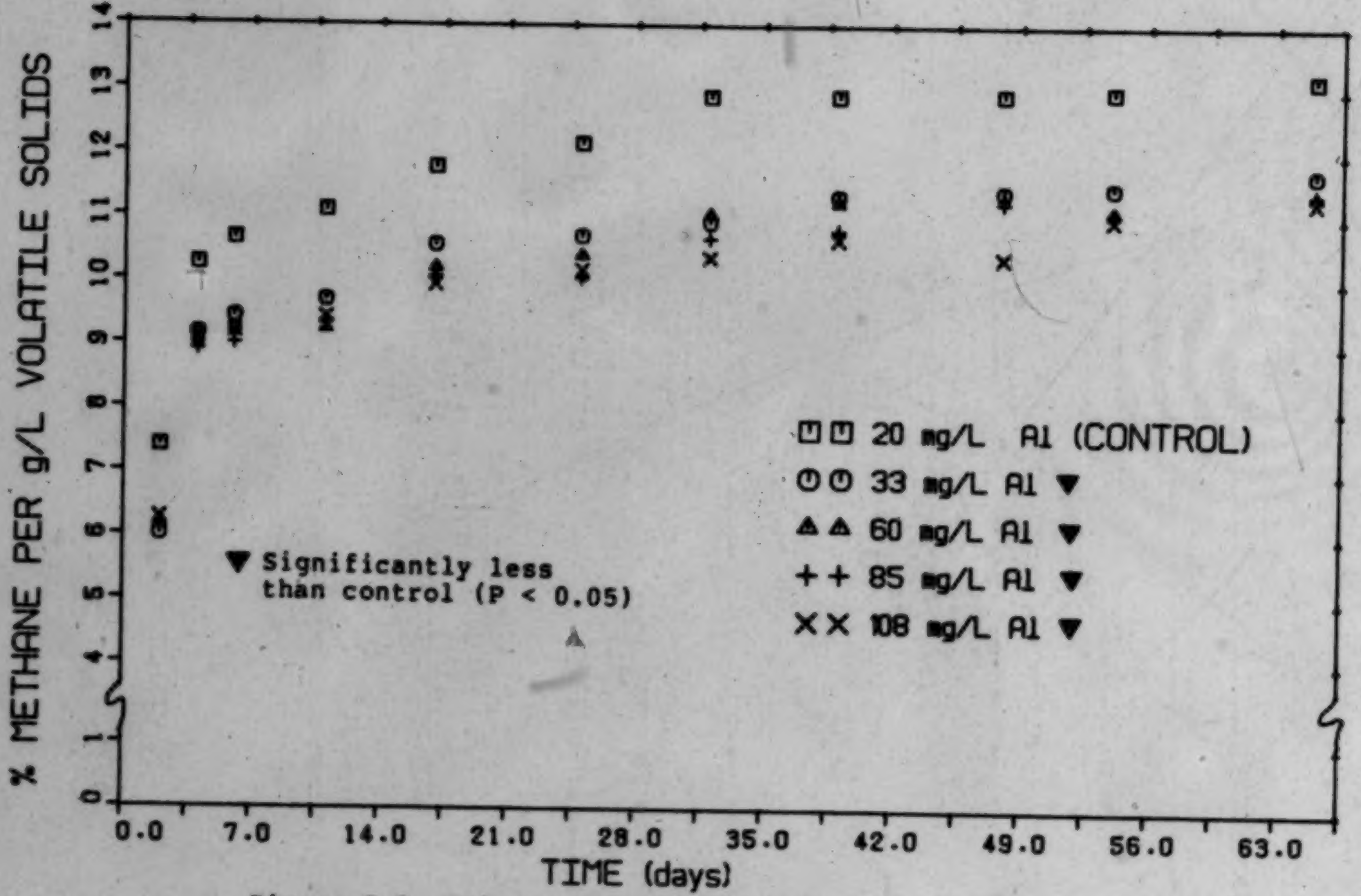

Figure 5.8 Bioassay Test - Alum Experiment R3 With Acetate Supplement

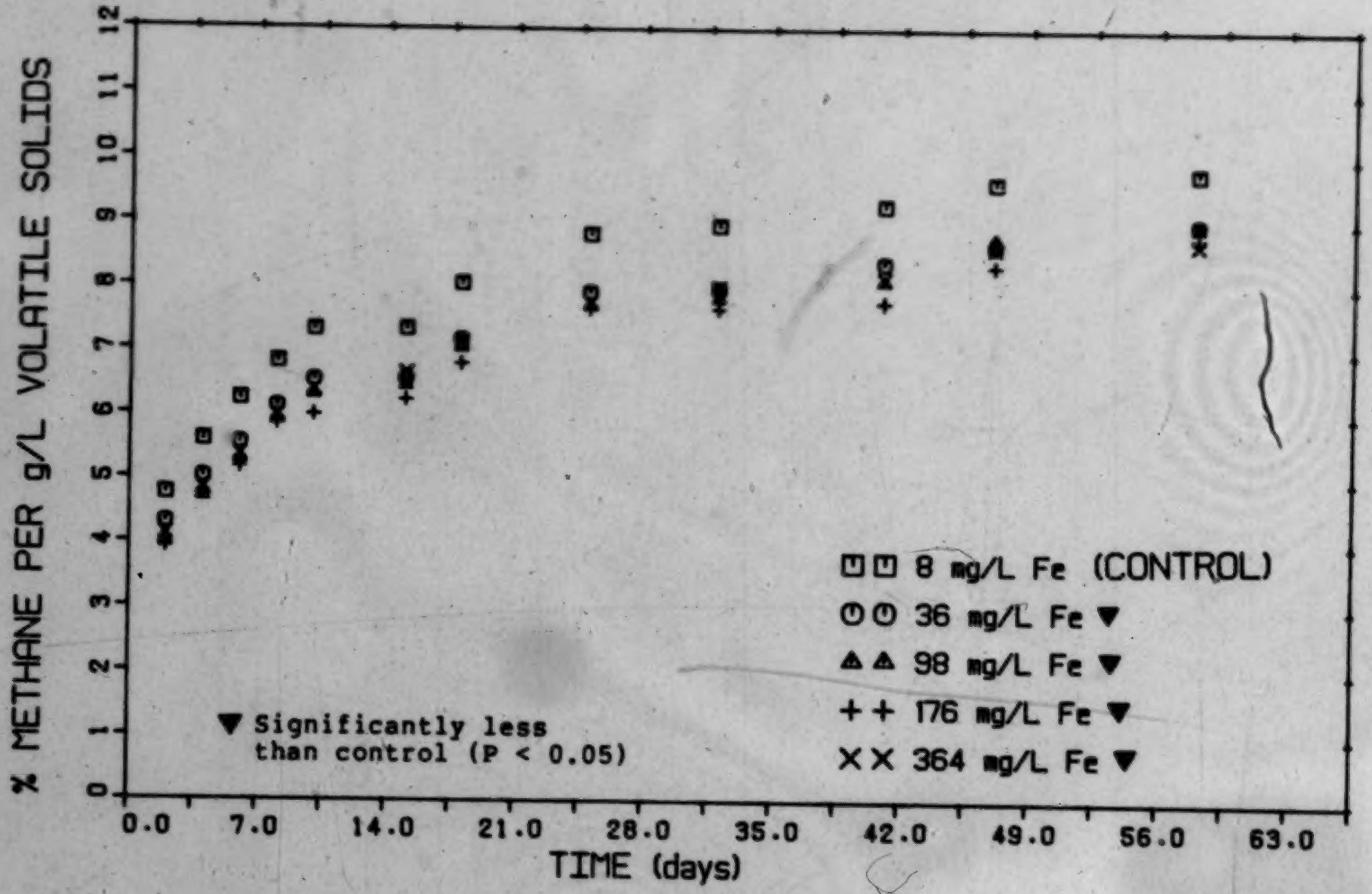

Figure 5.9 Bioassay Test - Ferric Chloride Experiment R3

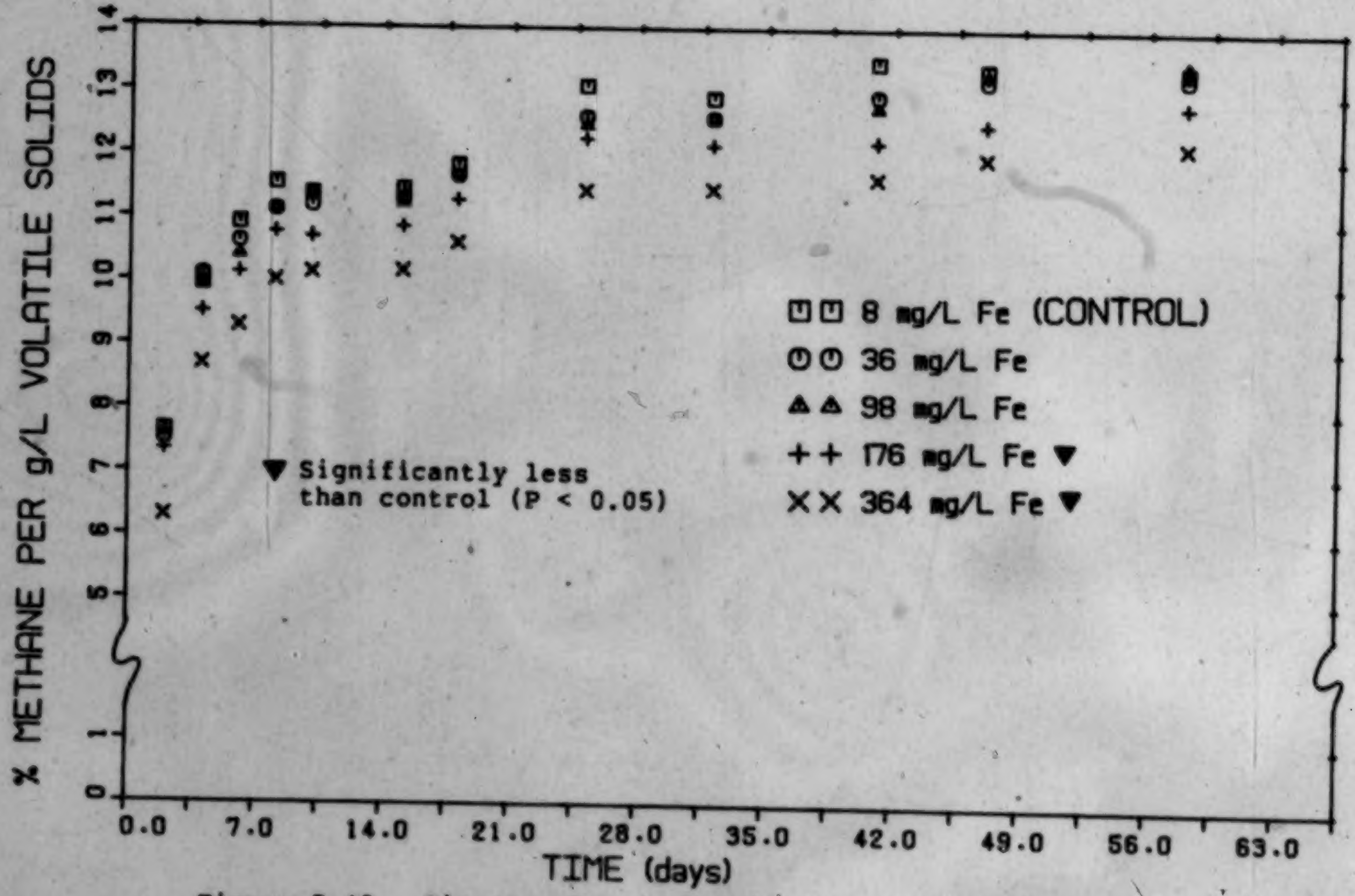

Figure 5.10 Bioassay Test - Ferric Chloride Experiment R3 With Acetate Supplement

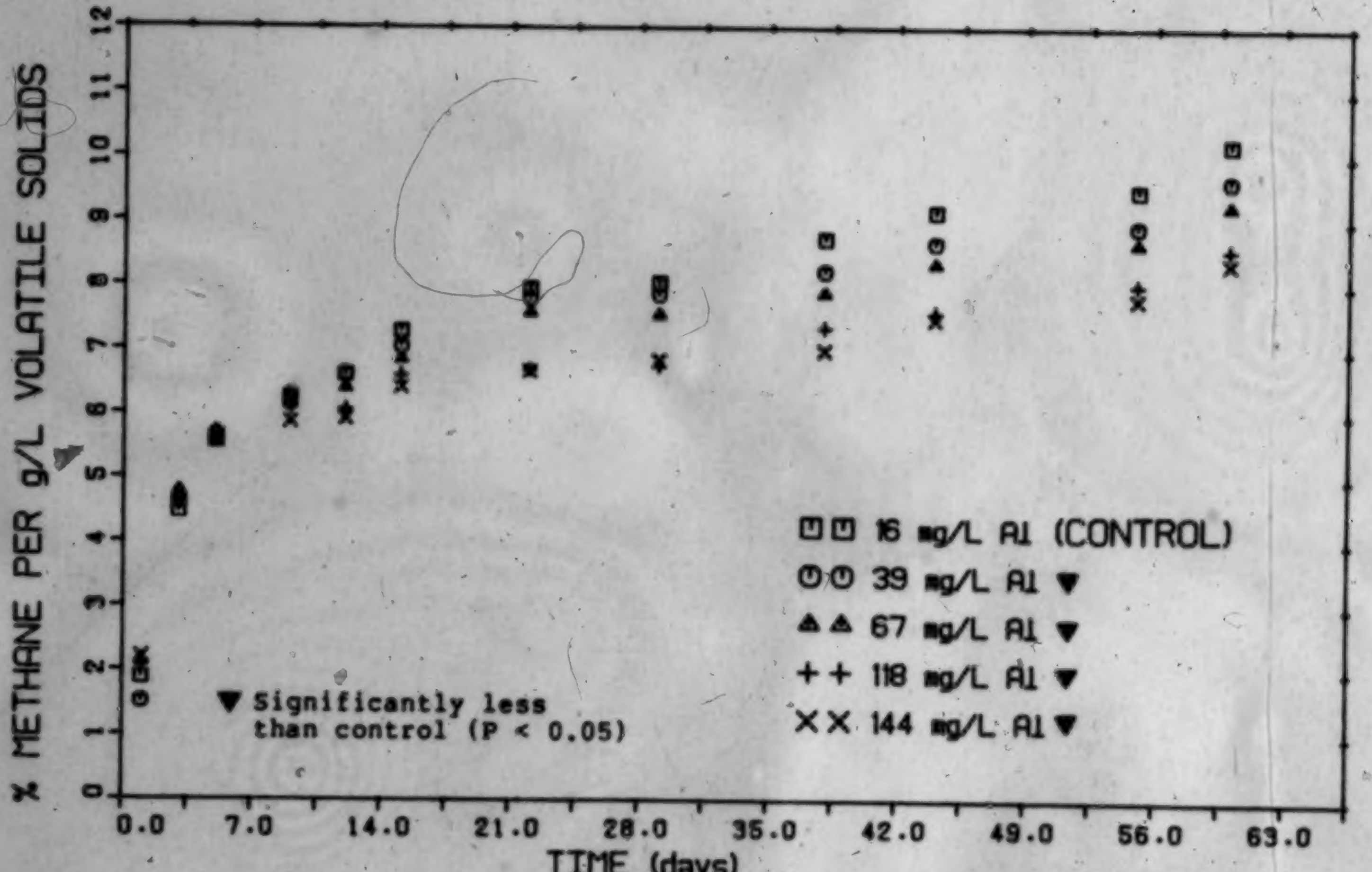

Figure 5.11 Bioassay Test - Alum Experiment R4

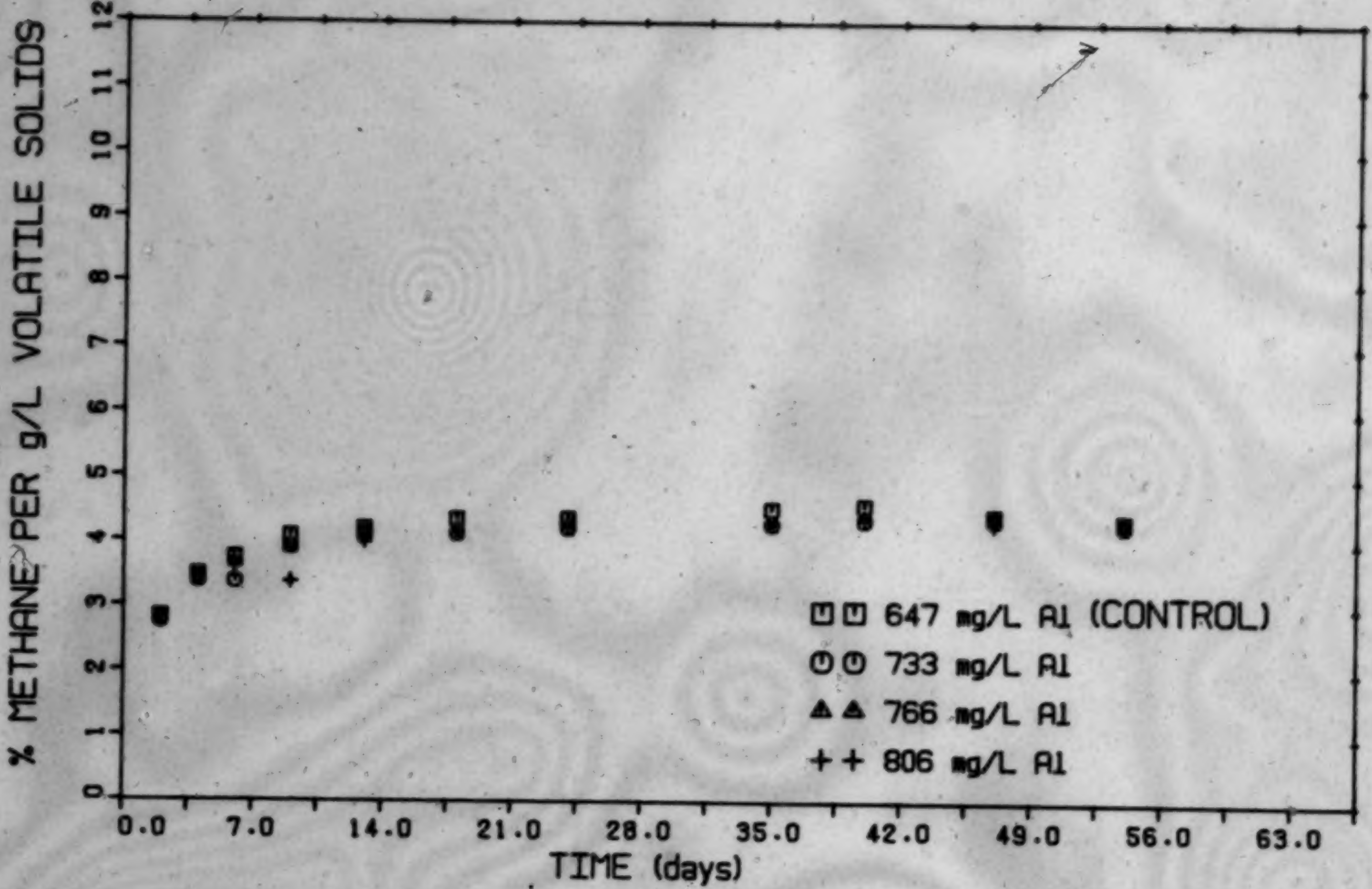

Figure 5.12 Bioassay Test - Alum Experiment R5

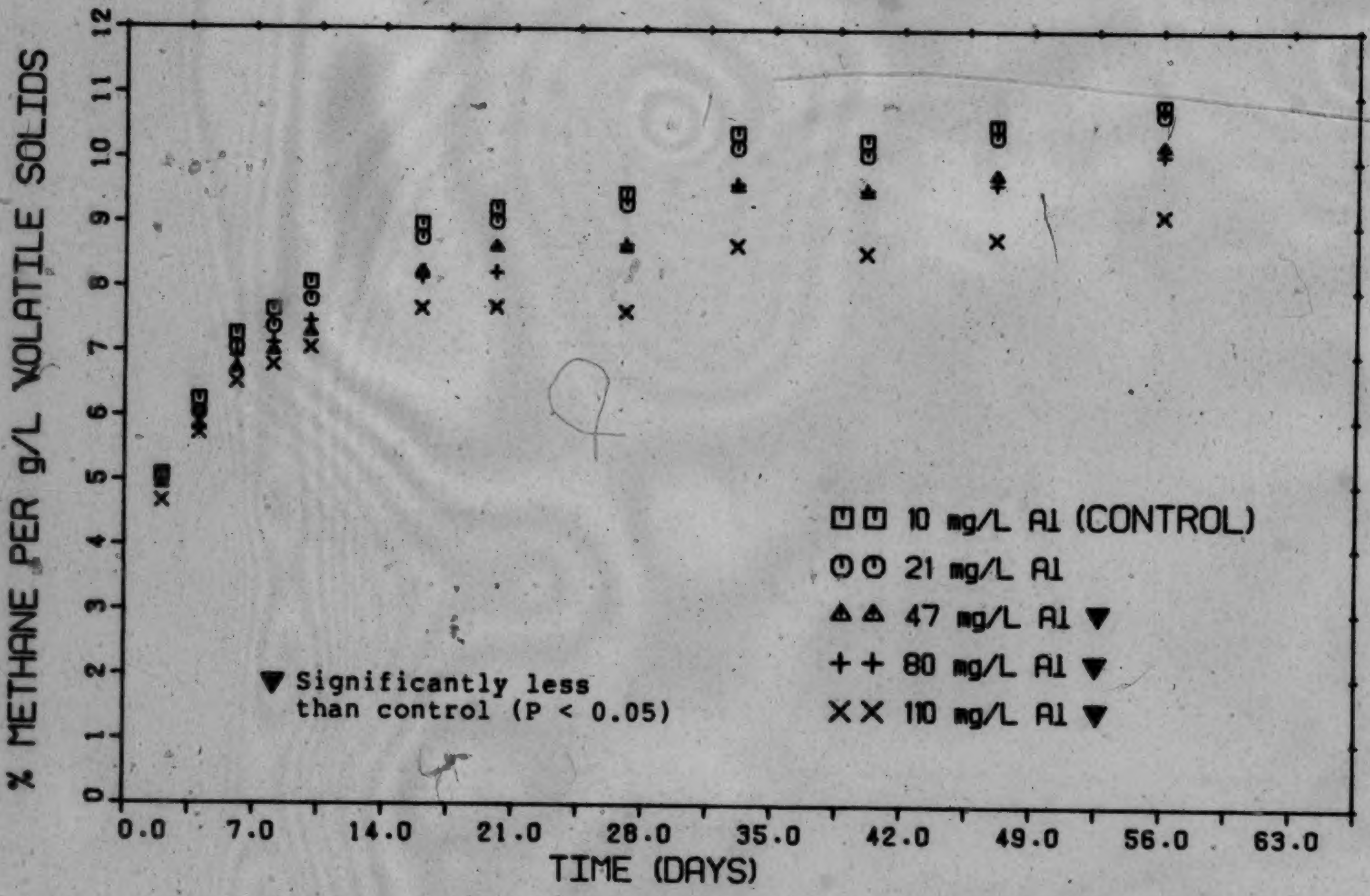

Figure 5.13 Bioassay Test - Alum Experiment R6

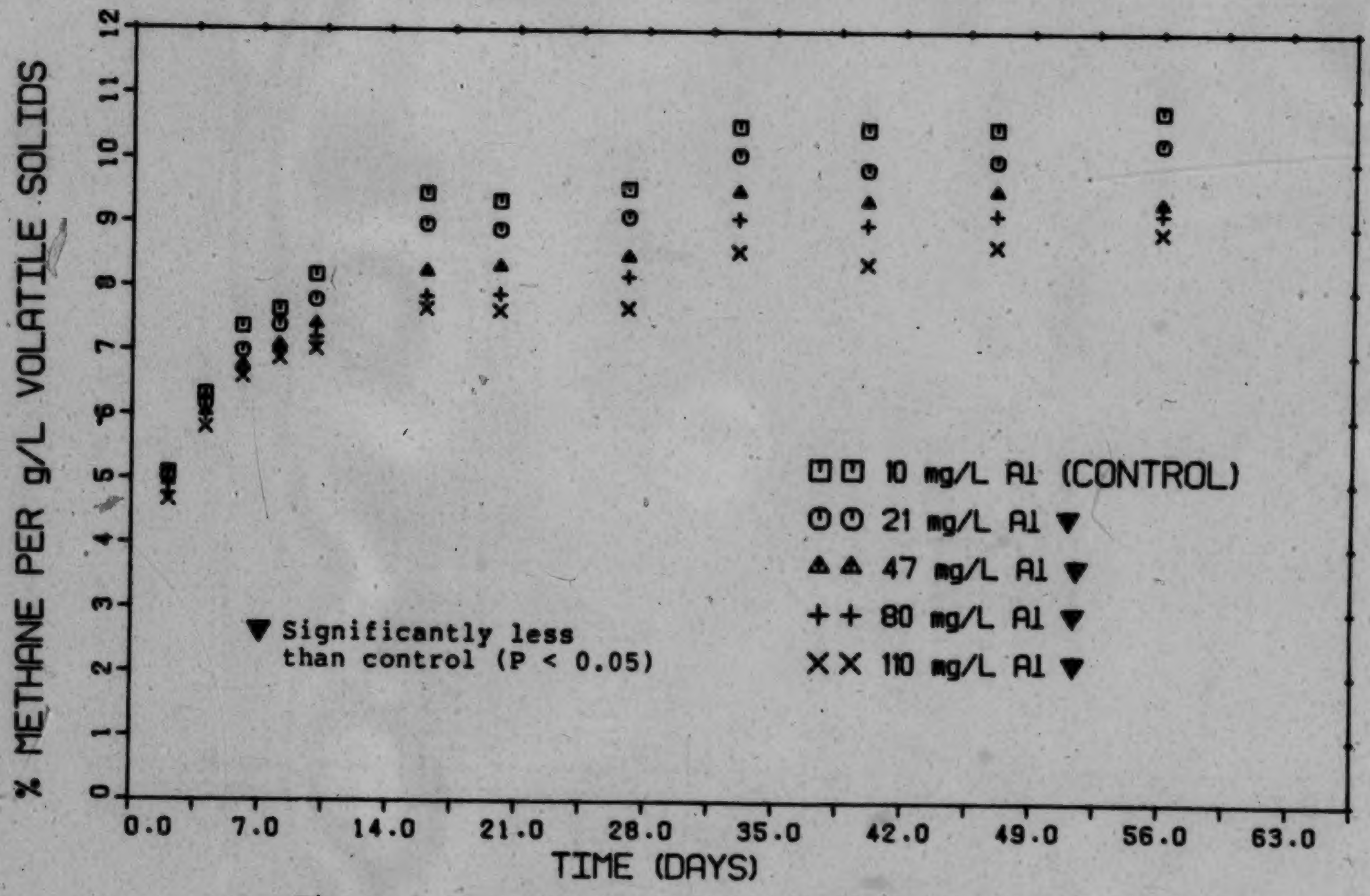

Figure 5.14 Bioassay Test - Alum Experiment R6
With Alkalinity Supplement

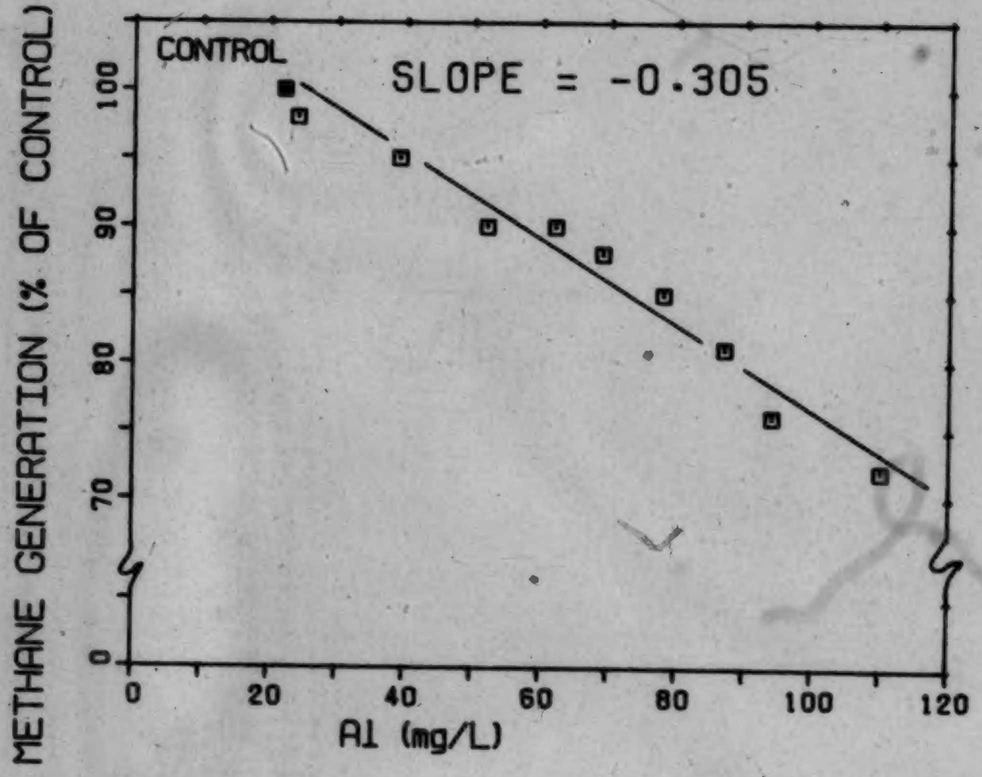

Figure 5.15 Methane Generation - Alum Experiment R1

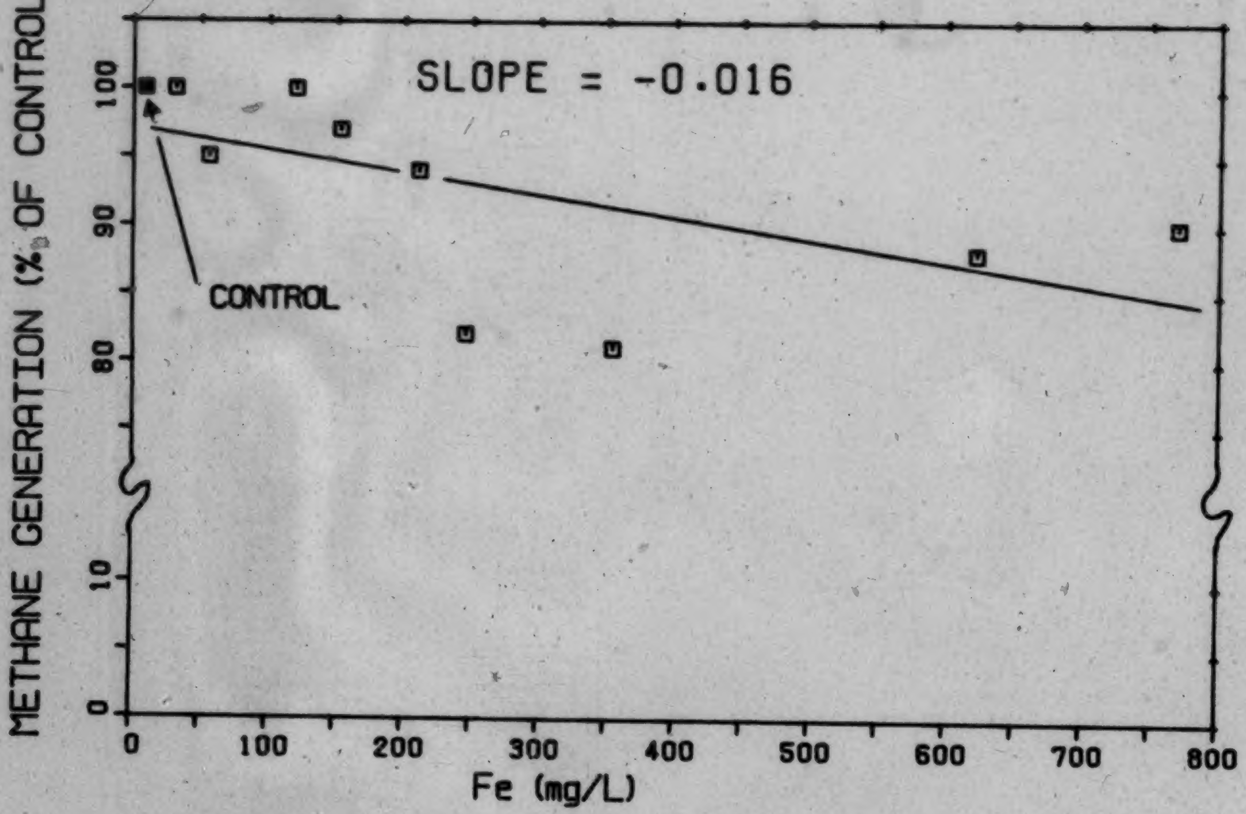

Figure 5.16 Methane Generation - Ferric Chloride
Experiment R1

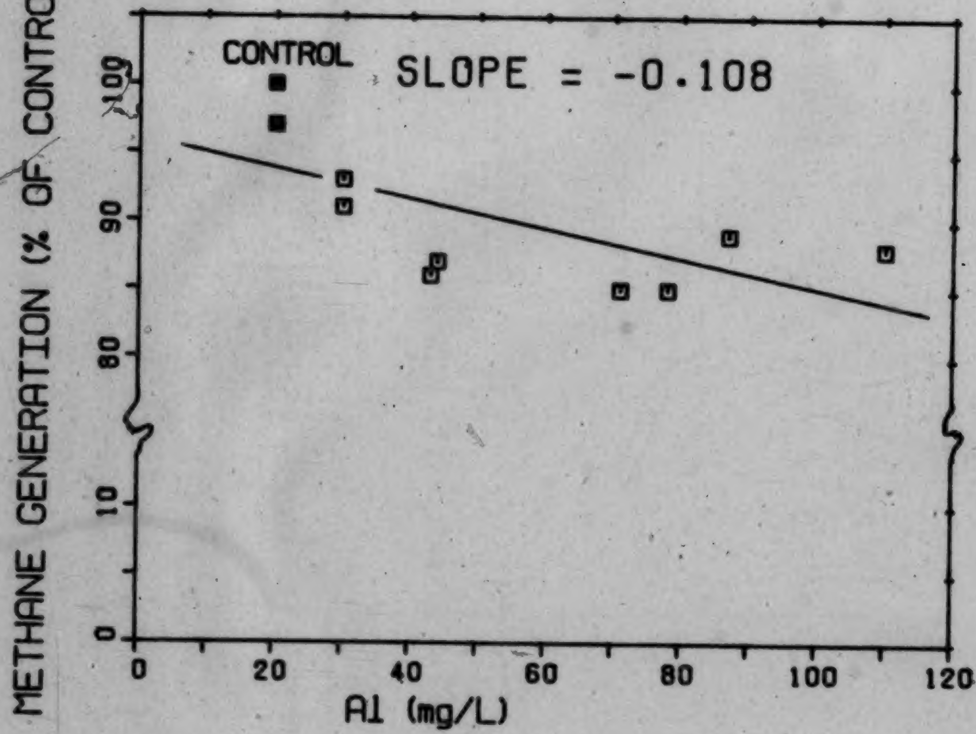

Figure 5.17 Methane Generation - Alum Experiment R2

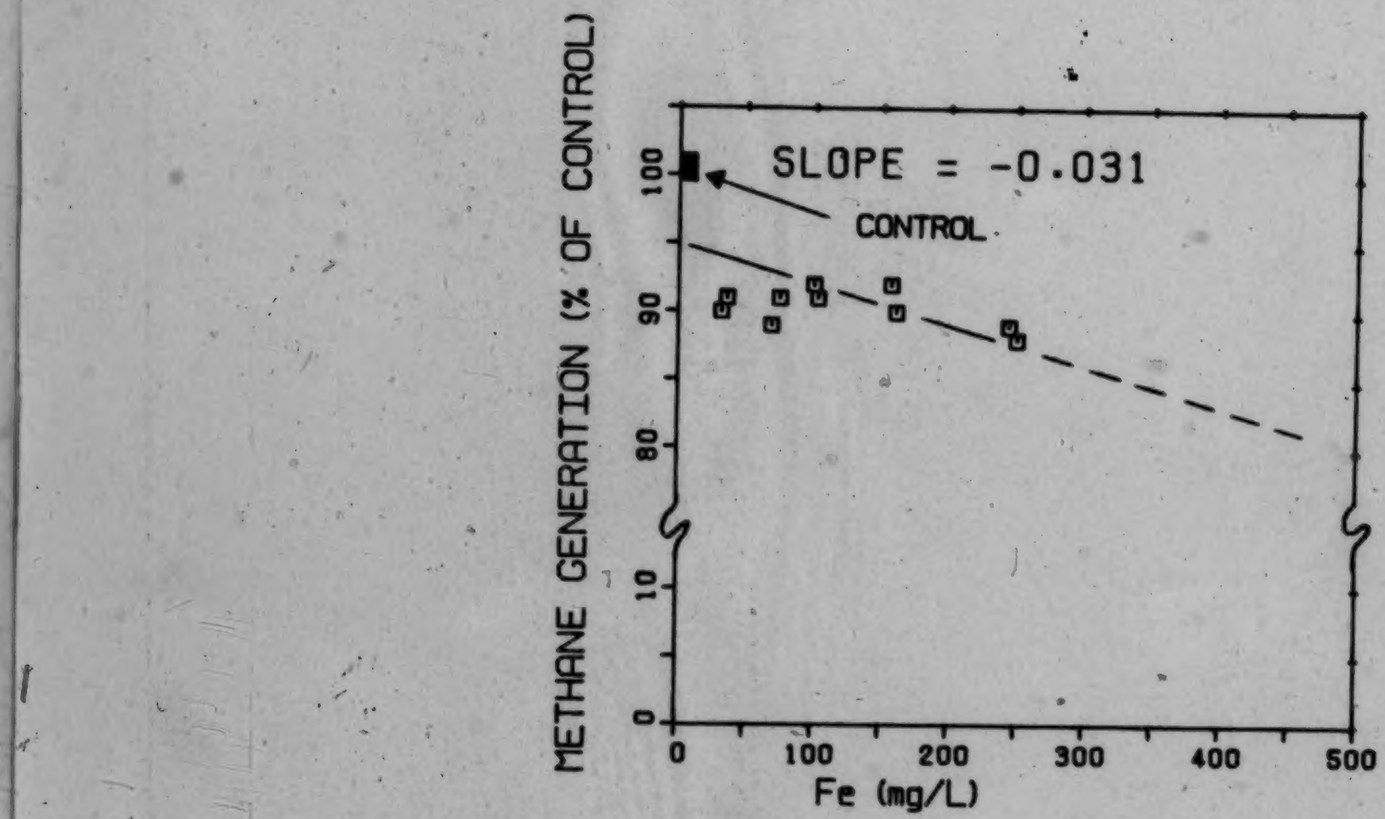

Figure 5.18 Methane Generation - Ferric Chloride Experiment R2

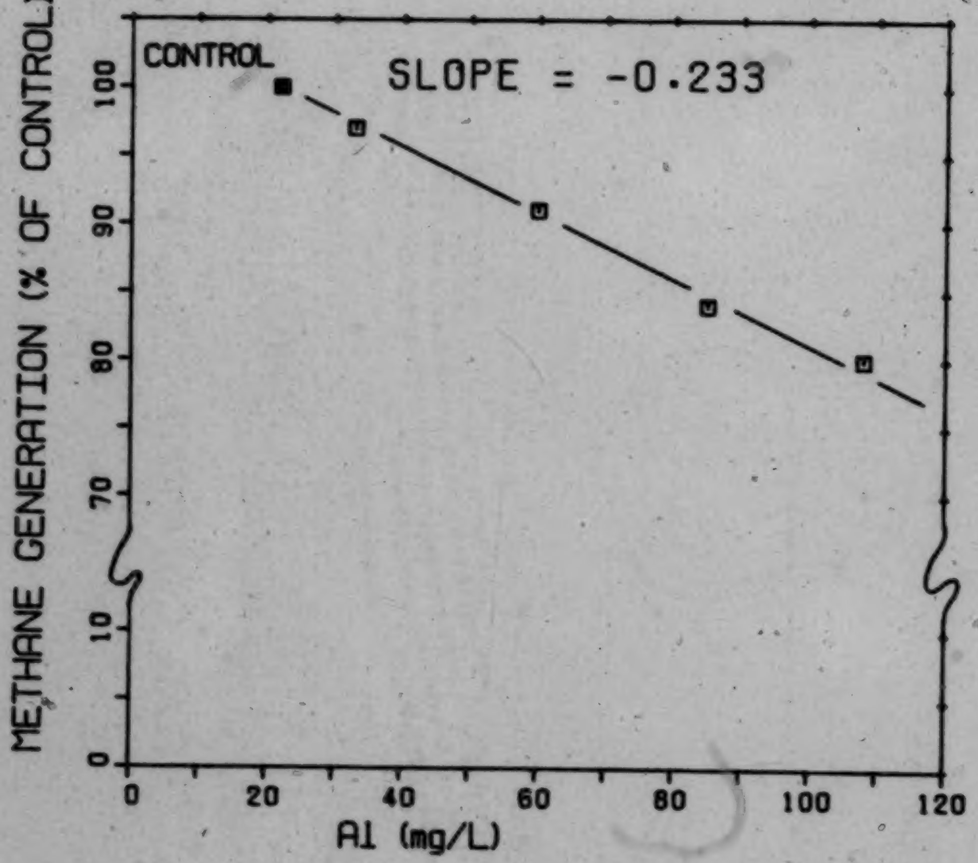

Figure 5.19 Methane Generation - Alum Experiment R3

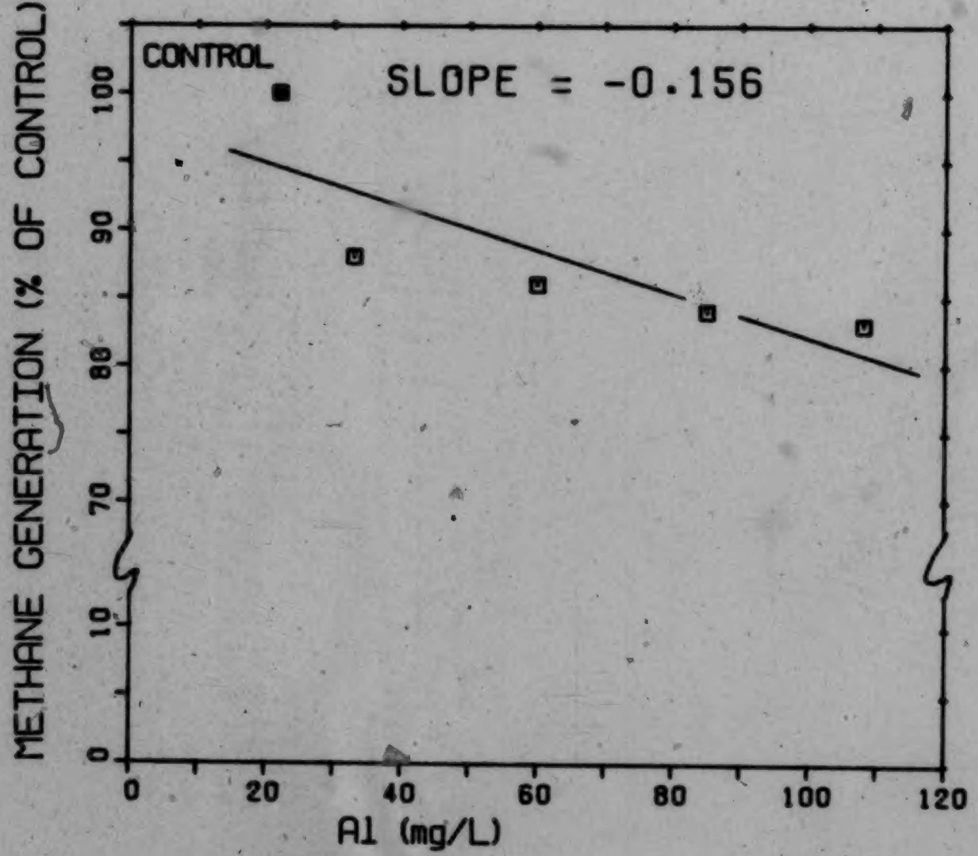

Figure 5.20 Methane Generation - Alum Experiment R3 With Acetate Supplement

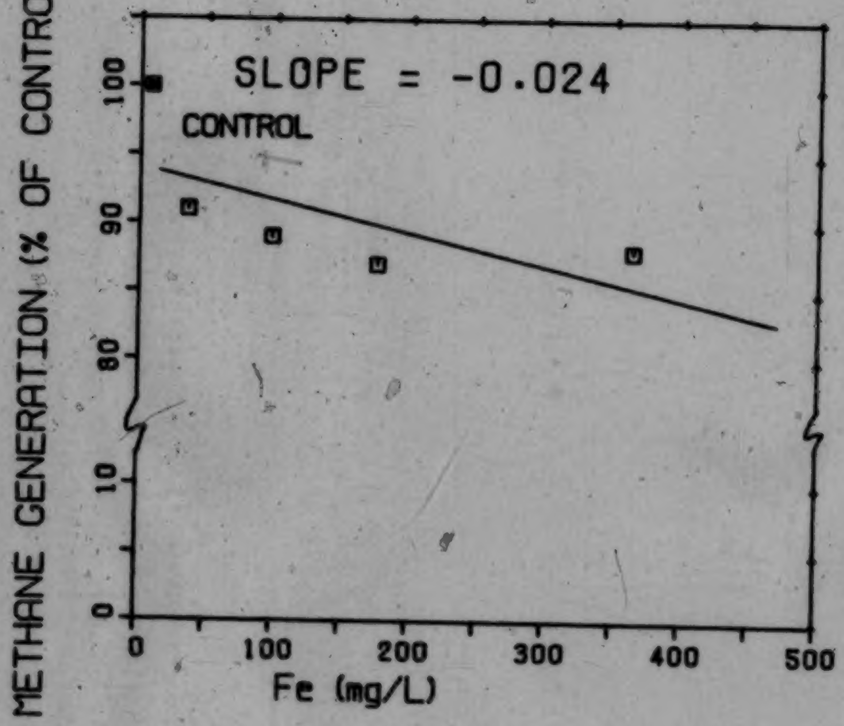

Figure 5.21 Methane Generation - Ferric Chloride Experiment R3

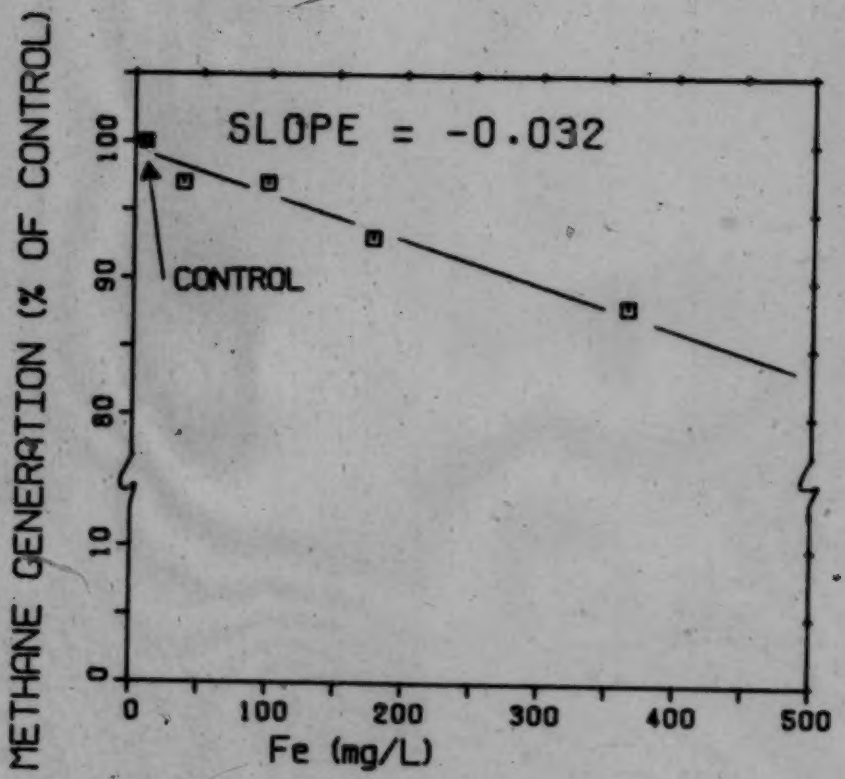

Figure 5.22 Methane Generation - Ferric Chloride
Experiment R3
With Acetate Supplement

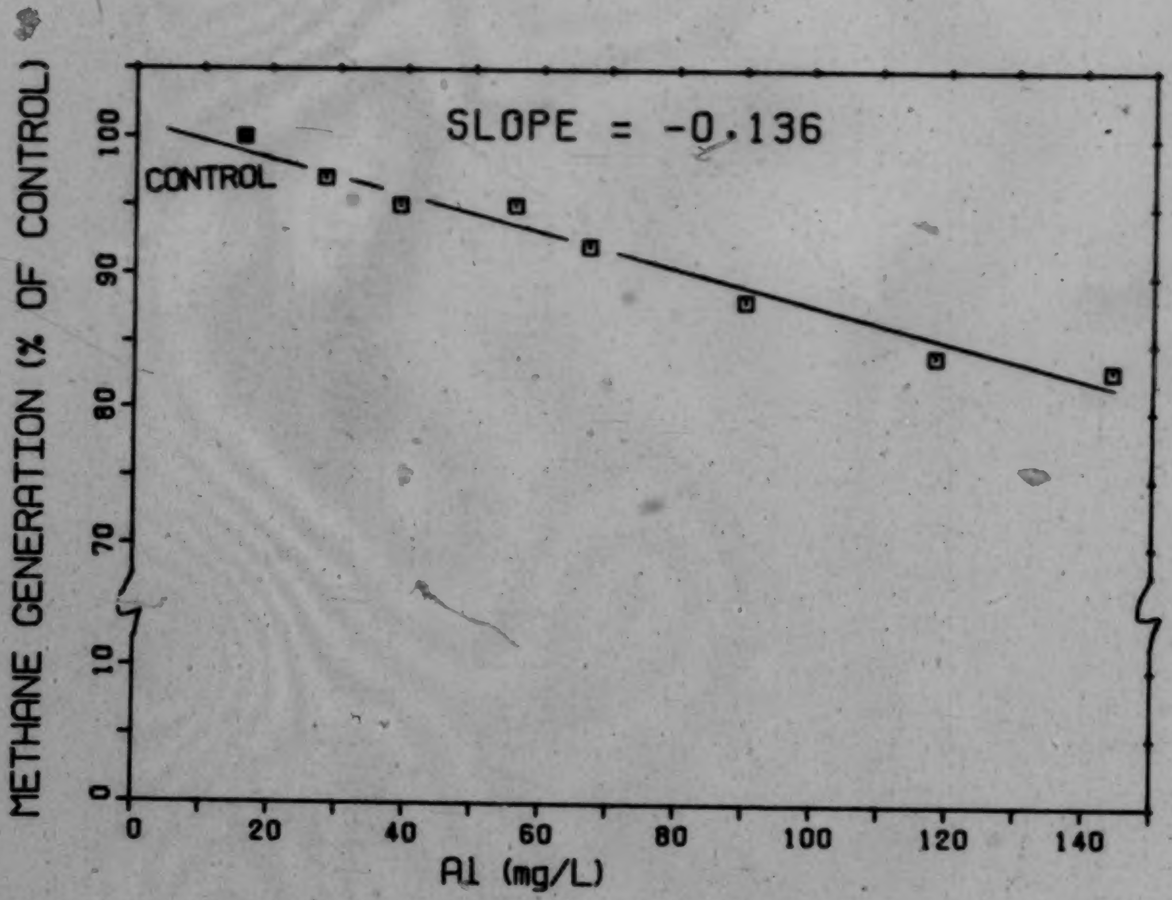

Figure 5.23 Methane Generation - Alum Experiment R4

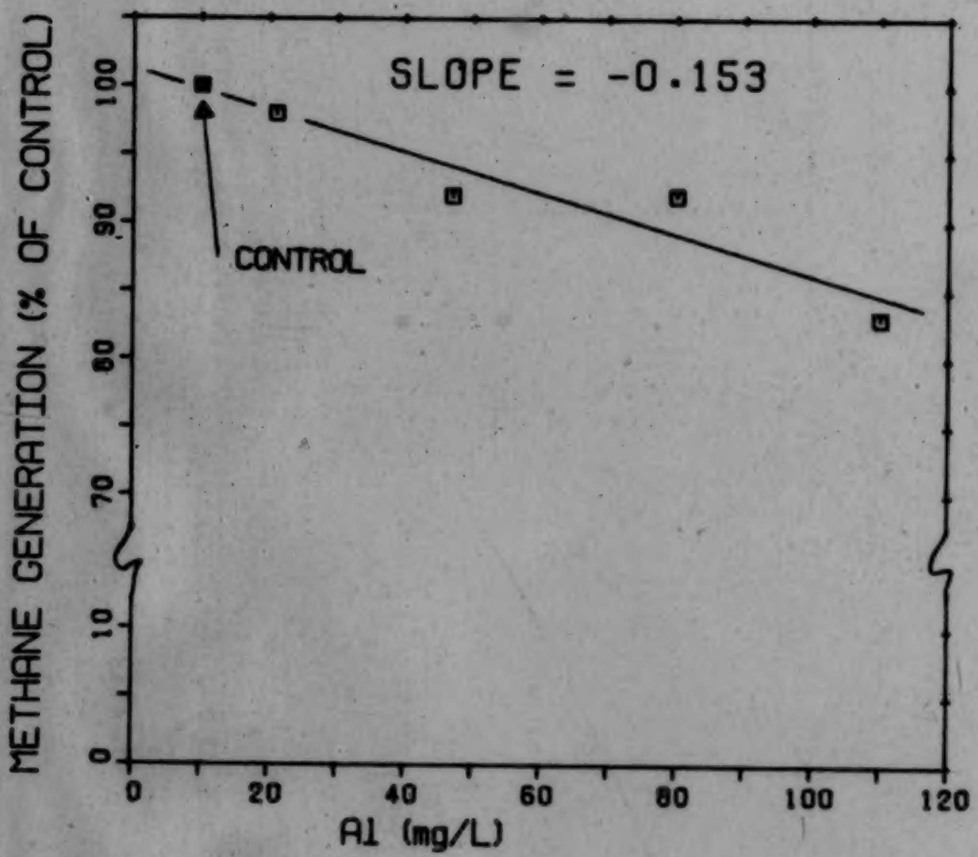

Figure 5.24 Methane Generation - Alum Experiment R6

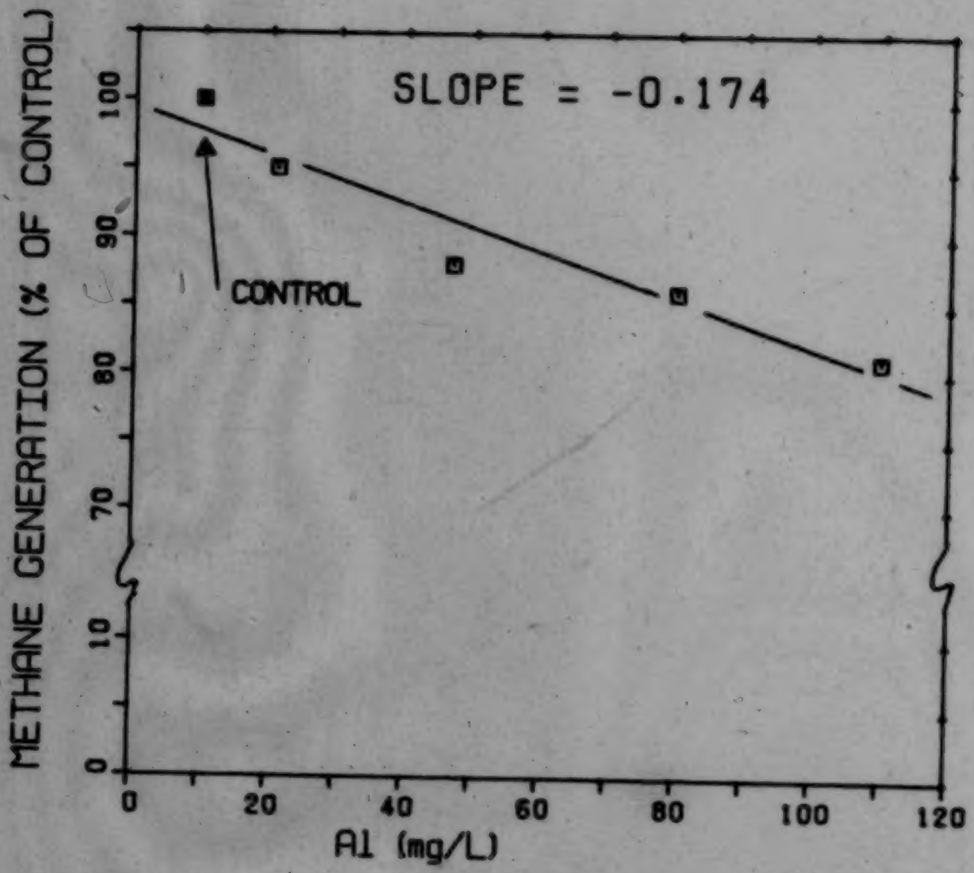

Figure 5.25 Methane Generation - Alum Experiment R6 With Alkalinity Supplement

Table 5.10 **Test For Significance - Alum Experiments**

Alum Experiment	**Slope ≠ 0'** **($P < 0.05$)**
R1	*Yes*
R2	*Yes*
R3	*Yes*
R3 With Acetate Supplement	*No*
R4	*Yes*
R6	*Yes*
R6 With Alkalinity Supplement	*Yes*

' Slope of linear aluminum dose methane response relationship.

NOTE: Results apply only to the range of aluminum concentrations tested in all experiments.

Table 5.11 **Test For Significance - Ferric Chloride Experiments**

Ferric Chloride Experiment	**Slope ≠ 0'** **($P < 0.05$)**
R1	*No*
R2	*Yes*
R3	*No*
R3 With Acetate Supplement	*Yes*

' Slope of linear iron dose methane response relationship.

NOTE: Results apply only to the range of iron concentrations tested in all experiments.

Table 5.12 Comparison of Methane Generation - Alum Experiment R3

	Al (mg/L)	Methane Generation' Without Acetate	Methane Generation' With Acetate
CONTROL	20	100	100
II	33	97	88
IV	60	91	86
VI	85	84	84
VIII	108	80	83

' % of control.

NOTE: Methane generation values are averages for three different incubation times shown in Tables III.5 and III.6

Table 5.13 Comparison of Methane Generation - Ferric Chloride Experiment R3

	Fe (mg/L)	Methane Generation'	
		Without Acetate	With Acetate
CONTROL	8	100	100
II	36	91	97
VI	98	89	97
V	176	87	93
VI	364	88	88

' % of control.

NOTE: Methane generation values are averages for three different incubation times shown in Tables III.7 and III.8

6. DISCUSSION OF RESULTS

6.1 Wastewater Characteristics

The following discussion relates to Tables 5.1 to 5.9. Control cultures for all the bioassays contained various baseline amounts of aluminum (alum experiments) and iron (ferric chloride experiments). Aluminum and iron in the control cultures were present in the activated sludge and anaerobic sludge obtained from the wastewater treatment plants since the control samples did not receive any chemical addition during sludge preparation. Aluminum concentrations ranged from 10 to 22 mg/L and iron concentrations of 7 to 9 mg/L were present in the control cultures. These values appear low when compared to finally digested sludge (400 mg/L Al or 800 mg/L Fe) from digesters where neither alum or ferric chloride addition was practiced (Department of Land Resource Science and Microbiology, University of Guelph; 1976) even when taking into account the expection of the higher values for finally digested sludge. The form in which the metals appear in the sludge samples is unknown (soluble or insoluble). Their presence represents a baseline for a municipal sludge source where chemical coagulant addition was not practiced. The effects caused by addition of chemical coagulant during sludge preparation are compared to this baseline.

Aluminum concentrations in all treatment conditions for Alum Experiment R5 (Table 5.8) were very high (647 to

806 mg/L). The sample sources contributed 647 mg/L (control) because they were taken from a plant practicing alum addition (Bonnybrook Wastewater Treatment Plant, Calgary). The high aluminum concentrations and total solids (Table 5.8) in the control is probably not representative of average activated sludge and is probably biased to high aluminum concentrations. In retrospect the method of sampling the activated sludge at Bonnybrook, discussed in section 4.1, probably resulted in samples that were not representative of the treatment process.

Alkalinity concentrations (mg/L as $CaCO_3$) are recorded for all treatment conditions prior to incubation. Alkalinities generally ranged from 900 to 1,200 mg/L. Although these concentrations appear low according to Figure 3.2, methane production was not adversely affected. This can be explained by examining alkalinity data for Alum Experiment R6 (Figures 5.1 and 5.2). Alkalinity concentrations increased 40 to 60% for all treatment conditions within 14 days of incubation (Figure 5.1). At no time were any decreases observed for the duration of the incubation period.

Figure 5.2 shows the results for all treatment conditions in Experiment R6 with the alkalinity supplement. Alkalinity concentrations initially ranged from 2,650 to 2,750 mg/L. Again, within 14 days the concentrations increased approximately 20% and did not decrease for the duration of the incubation period.

The increases in alkalinity during incubation may, in part, have originated from the biological degradation of organic nitrogen materials, as was discussed in section 3.2.2.1. This can be demonstrated by comparing the measured alkalinity concentrations with methane production (per g/L VS) in Alum Experiment R6 (Figures 5.1 and 5.13) and Alum Experiment R6 with the alkalinity supplement (Figures 5.2 and 5.14). The increases in alkalinity coincides with the increases in methane production in the early stages of incubation (i.e. increased alkalinity production corresponds to an increasing degree of volatile solids destruction). Alkalinity concentrations then level out similar to the methane production curves. By this time the majority of the substrate has been digested and little alkalinity is being produced or utilized in a buffering capacity. Theoretical calculations on alkalinity production from ammonia released during catabolism of organic nitrogen materials for Alum Experiment R6 are presented in Appendix I.

The presence of aluminum did not have any adverse effects on alkalinity production for all treatment conditions tested in Alum Experiment R6 because no decreases were observed. This contradicts work performed by Gossett et al. (1978) where lower alkalinity concentrations were observed for higher alum dosages. However it should be noted that Gossett et al. performed semi-continuous digestion experiments while all bioassays for this work were batch experiments.

6.2 Bioassays

6.2.1 Alum Experiments

Batch digestion of culture containing aluminum precipitated sludge show decreasing methane production, on a "per mass of volatile solids loading" basis (Figures 5.3, 5.5, 5.7, 5.11, and 5.14). The observed methane concentrations in the serum bottle headspace decreased with increasing aluminum concentrations suggesting that the decreased methane production occurred from the presence of aluminum. Most of the activity (substrate metabolism and methane production) occurred during the first 14 to 21 days of incubation. This is observed by the sharp increases in methane concentrations (e.g. Figures 5.3 and 5.5). A trend developed between methane concentrations and the levels of aluminum present during this period (i.e. a reduced level of methane production for a given aluminum concentration). After 21 days of incubation the trend remained "consistent" although minor increases in methane concentrations were observed for all treatment conditions (Figures 5.5, 5.7, and 5.13). This suggests that some activity was still occurring at a very low rate, however the nature of the effect that aluminum had on methane production did not lessen for the total incubation period (55 to 60 days).

Hypothesis proposed to explain the nature of the effect chemical coagulants have on the anaerobic digestion process include (Gossett et al., 1979):

1. chemical coagulant toxicity;
2. changes in sludge organic composition induced by coagulation;
3. phosphorus limitation; and
4. interference with enzymatic hydrolysis (discussed in section 3.3.2).

Another mechanism that may affect digestion of the aluminum precipitated sludge is the presence of sulphates (SO_4^{2-}).

1. Chemical coagulant toxicity.

Results of the batch digestion experiments with aluminum precipitated sludge do not support the theory of aluminum exhibiting toxic effects on the anaerobic microorganisms. The sudden introduction of large concentrations of aluminum to the anaerobic bacteria should produce immediate adverse effects if the aluminum is toxic. This did not appear to be evident as cultures containing 110, 108, and 144 mg/L Al (Figures 5.5, 5.7, and 5.11 respectively) produced methane at approximately the same rate as the control cultures in the first 3 to 5 days of incubation in each bioassay. This is demonstrated by the similar slopes in methane production rates for each culture.

2. Changes in sludge organic composition.

Chemical coagulation aids in the removal of colloidal materials from wastewater. A decrease in the biodegradability of organics might be observed if the colloidal materials were less biodegradable than the settleable organic solids.

The volatile solids loading for each treatment condition in Alum Experiment R6 reflects the effect of added colloidal materials from alum coagulation, along with the settleable organic fraction (Table 5.9). Recall from section 4.1, that during sludge preparation the aluminum precipitated activated sludge samples for all treatment conditions were adjusted to the same settled sludge volume by removing an equivalent amount of supernatant from each cylinder. The net result was that the volume of sludge in each cylinder contained the typical settleable fraction of organics, plus any additional colloidal organics the given dose of alum coagulated from the activated sludge. This amount of additional colloidal organics is reflected in Table 5.9 (taking into account the contribution of organics from the anaerobic seed sludge). The volatile solids loading for the culture containing 110 mg/L of Al was approximately 5% greater than the volatile solids loading in the control culture. It does not seem likely that this marginal increase in volatile organics (from additional colloidal materials) could "fully" account for the observed difference in %CH_4 per g/L VS between the culture containing 110 mg/L Al and the control (Figure 5.13). Findings by Gossett et al. (1979) support this statement. However, it may be possible that these effects act in addition to the other mechanism(s) to produce the overall adverse effect.

3. Phosphorus limitation.

When anaerobic microorganisms release inorganic phosphates (PO_4^{3-}) during decomposition of organic phosphorus compounds, precipitation reactions are likely to occur between chemical coagulants and the orthophosphate anions. This phenomenom could be occurring in the culture bottles thereby limiting the microorganisms of the nutritionally essential phosphorus. Gossett et al., (1979) reported that the possible effects caused by phosphorus limitation could not "fully" account for the observed chemical coagulant effects on anaerobic digestion. However, phosphorus limitation cannot be ruled out completely and it may be posssible that these effects act in addition to other mechanism(s) to produce the overall adverse effect.

4. Interference with enzymatic hydrolysis.

The chemical coagulation process may render volatile solids less accessible and/or less reactive to the extracellular enzymes of the acid forming bacteria during the digestion process (the physical exclusion phenomenom discussed in section 3.3.2). This mechanism might allow the acid forming bacteria to metabolize the organics that were unaffected during the coagulation process, but it would prevent or retard any metabolism of the organics that were affected (by physical exclusion). The chemical coagulant floc that forms during coagulation may provide an effective barrier to the extracellular enzymes of the acid forming bacteria during digestion (Gossett et al., 1979). The extent or degree to which the organics are physically excluded from being metabolized by the acid forming bacteria would be apparent during the latter stages of incubation. The control culture should have a greater amount of organics metabolized, and therefore, show a higher methane concentration than the alum dosed cultures near the end of the incubation period. The methane concentration for any given treatment condition was "consistently" below the control after 21 days of incubation (Figures 5.3, 5.5, and 5.7). This strongly suggests that a reduced degree of substrate digestion was occurring for the alum dosed cultures.

The duration of the incubation period (55 to 60 days) did not appear to be long enough for the bioassays to determine if the physical exclusion phenomenom could eventually halt methane production in the dosed cultures. Methane concentrations for all treatment conditions in the bioassays did not completely level off at any time. However, the methane concentration for any given alum dosed culture was "consistently" below that of the control. The fact that methane concentrations from the alum dosed cultures did not increase over time with respect to the control supports the physical exclusion phenomenum.

5. The presence of sulphates (SO_4^{2-}).

Alum coagulation of wastewater increases the sulphate concentration (see section 3.1.1) and, along with sulphates originally present in the untreated wastewater, may affect subsequent anaerobic digestion.

A manner in which sulphates can exhibit inhibition of methane formation is as a result of a species compositional change (Zeikus, 1979). Sulphate-reducing bacteria appear to compete more effectively for hydrogen gas and acetate than the methanogenic bacteria in the presence of high sulphate concentrations. This condition increases the sulphate-reducing population at the expense of the methanogenic bacteria. As a result, increased H_2S production and decreased CH_4 formation can occur.

The possibility of sulphates being a precursor to soluble sulphide toxicity may also adversely affect subsequent anaerobic digestion (Lawrence et al., 1964). Concentrations of soluble sulphide (S^{2-}) greater than 100 mg/L have been reported to cause moderate inhibition in digesters (McCarty, 1964b). Calculated soluble sulphide concentrations from addition of the highest alum dose (2,000 mg to 2 L of activated sludge; see Table 4.1) could be as high as 50 to 80 mg/L (see Appendix V). The combined effect of this concentration of soluble sulphide and soluble sulphide conversion from sulphates originally present in the wastewater may be significant enough to exert moderate inhibition in test cultures with the highest aluminum concentrations.

It would appear that the effect of sulphates may be important and could act in addition to the other mechanism(s) to produce an overall adverse effect for some of the test cultures containing high aluminum concentrations.

Methane concentrations for all treatment conditions in Alum Experiment R3 with the acetate supplement increased very rapidly in the first few days of incubation (Figure 5.8). The acetate utilizing methanogens appeared to readily consume the acetate supplement during this period in all cultures. The trend in methane production for this bioassay was different compared to the characteristic trends observed in the other alum bioassays. Cultures dosed with alum showed rapid onset of a differential in methane concentrations compared to the control. This was not observed in the other alum bioassays (e.g. Figure 5.3, 5.5, and 5.7).

Batch digestion results for sludge obtained from the treatment plant where alum addition is practiced (Bonnybrook) are shown in Figure 5.12 (Alum Experiment R5). Methane concentrations, on a "per mass of volatile solids loading" basis, were similar for all treatment conditions for the whole incubation period. These methane concentrations were low compared to values observed for other alum bioassays (e.g. Figure 5.3). The control culture in Alum Experiment R5 cannot be considered a "control" in the same sense as in the other bioassays to which "test cultures" dosed with alum were compared to assess the effects of aluminum. The low methane production from the control (already containing 647 mg/L Al) probably suggests that the mechanism(s) responsible for the adverse effects continues to increase in severity from the aluminum concentrations tested in the other alum bioassays to the concentrations tested in this bioassay. The fact that the observed methane concentrations for all treatment conditions in Alum Experiment R5 did not differ significantly suggests a plateau effect was occurring (with respect to the nature of the chemical effect) at the high aluminum concentrations tested (647 to 806 mg/L Al). The "true" magnitude of the adverse chemical effect at which the plateau effect was observed is unknown.

6.2.2 Ferric Chloride Experiments

Batch digestion of cultures containing iron precipitated sludge showed conditions of decreased methane production, on a "per mass of volatile solids loading" basis, however no consistent trends were observed with increasing iron concentrations (Figures 5.4, 5.6, and 5.9).

Methane concentrations for all treatment conditions in Ferric Chloride Experiment R3 with the acetate supplement increased rapidly in the early stages of incubation (similar to the alum bioassay with the acetate supplement) (Figure 5.10). Only iron concentrations greater than 176 mg/L were shown to cause significant adverse effects in methane production ($P < 0.05$). The evidence by Speece (1983) that iron is a necessary trace nutrient for acetate utilizing methanogens suggests that these organisms may have considerable tolerance to iron. Iron concentrations of 36 and 98 mg/L did not produce any significant adverse effects in methane production.

6.3 Coagulant Dose Methane Response Relationship

6.3.1 Alum Experiments

The significance tests that were performed on methane generation from the alum bioassays demonstrated a linear relationship may exist between aluminum concentrations and methane production (Table 5.10). Methane generation decreased consistently for increasing aluminum concentrations (e.g. Figures 5.15, 5.19, 5.23, 5.24, and 5.25). Overall this effect was observed for increasing aluminum concentrations up to 144 mg/L (Figure 5.23).

Although the significance tests were performed assuming a linear relationship, some of the relationships were non-linear (Figures 5.17 and 5.20). The slopes of the linear relationships differed for each bioassay also. The nature of the methane generation relationship observed (i.e. linear or non-linear) and the variability in slopes of the linear relationships can best be attributed to the physical exclusion phenomenum discussed in section 6.2.1. Different settling characteristics were observed for each treatment condition during sludge preparation. This was probably due to the type or nature of the floc that formed during the actual coagulation process. Since the nature of the floc formation taking place during the coagulation process can result in different types of floc being formed, it could affect the degree to which the organics are less accessible to enzymatic hydrolysis by the acid forming bacteria. Settling characteristics such as pin point floc were observed in some experiments and not in others for the same concentration of alum added during sludge preparation. These different settling characteristics (attributed to the characteristic floc formation) may help explain the differences in methane generation observed in various bioassays for treatment conditions containing similar aluminum concentrations. It would also help to explain the nature of the relationship and variability in the slopes of the linear relationships.

The types of floc formation that result in organics being "tied up" to a greater degree during coagulation, and therefore "less accessible" to the anaerobic microorganisms during digestion, may explain the reduced methane generation observed at the higher alum dosages. Greater amounts of floc formation occur at these dosages and the extent to which the organics are "tied up" is likely to be greater than at the lower alum dosages. As a result, less methane formation might be observed in the cultures containing greater amounts of aluminum because less organics are readily accessible to the acid forming bacteria.

Methane generation for the acetate supplemented cultures (Figure 5.20) reflects the differential in methane production between the cultures dosed with alum and the control culture, as was discussed in section 6.2.1. Comparisons of methane generation for the alum dosed cultures with and without the acetate supplement suggest that aluminum affected the acetate utilizing methanogens in an inconsistent manner (Table 5.12). Treatment conditions containing 33 and 66 mg/L Al had lower methane generation for the acetate enriched cultures than for the cultures without acetate. This observation was reversed at higher Al concentrations (108 mg/L). An analysis was performed on data from the last day of the bioassay (day 65) to estimate methane production from conversion of the acetate supplement only (Appendix VI). The expected methane volumes are compared to the measured volumes in Table 6.1. No major differences were evident with the expected and measured volumes. The fact that no dramatic differences were observed in the measured methane volumes for all treatment conditions indicates that most of the supplement was probably metabolized by the acetate utilizing methanogens. This suggests that aluminum probably did not adversely affect the acetate utilizing methanogens. This supports the findings of Gossett et al., (1979) that the nature of the chemical effect occurs with the non-methanogens.

Table 6.1 **Methane Production From Acetate Supplement**
Alum Experiment R3
(see Appendix VI)

ALUM EXP. R3	Al (mg/L)	MEASURED VOLUME CH_4 (mL)	EXPECTED VOLUME CH_4 (mL)
CONT.	20	9.0	9.8
II	33	8.1	9.2
IV	60	8.2	9.4
VI	85	10.1	9.5
VIII	108	9.9	9.8

6.3.2 Ferric Chloride Experiments

The significance tests that were performed on methane generation data from the bioassays did not conclusively demonstrate a linear iron dose methane response relationship (Table 5.11). Ferric Chloride Experiments R1, R2, and R3 demonstrated reduced methane generation for cultures dosed with ferric chloride, however the magnitude of the effect did not increase with increasing iron concentrations up to 770 mg/L (Figures 5.16, 5.18 and 5.21). A line with 0 slope would fit the data well in Figures 5.18 and 5.21 for the cultures dosed with feric chloride (i.e. excluding the control).

Similar to the alum experiments, the different settling characteristics that were observed for each treatment condition during sludge preparation (due to different types of floc formation) could help explain the variability in observed methane generation for the ferric chloride bioassays.

Methane generation for the ferric chloride dosed cultures that were supplemented with acetate were better than for the cultures without acetate (Table 5.13). Treatment conditions containing 36, 98, and 176 mg/L Fe respectively had higher methane generation for the acetate supplemented cultures. An analysis, identical to what was performed for Alum Experiment R3 enriched with acetate, will estimate methane production from the acetate supplement for the ferric chloride experiment. The calculated methane volumes are compared to the measured volumes in Table 6.2. The fact that the measured methane volumes for all treatment conditions dosed with ferric chloride were consistently above the volume for control indicates that most, if not all, of the acetate supplement was metabolized by the acetate methanogens. This suggests that the presence of iron did not adversely affect the performance of the acetate utilizing methanogens.

Table 6.2 Methane Production From Acetate Supplement
Ferric Chloride Experiment R3
(see Appendix VI)

FERRIC CHLORIDE EXP. R3	Fe (mg/L)	MEASURED VOLUME CH_4 (mL)	EXPECTED VOLUME CH_4 (mL)
CONT.	8	8.5	10.1
II	36	9.8	10.1
IV	98	10.4	10.0
VI	176	9.7	10.1
VIII	364	10.0	9.5

7. CONCLUSIONS

Alum addition to wastewater produced sludge that, when batch digested anaerobically, demonstrated reduced methane production. The magnitude of the adverse effects on methane production increased with increasing aluminum concentrations up to 144 mg/L. The type or nature of chemical floc that forms during the actual coagulation process may play an important role in influencing the magnitude of the adverse effects during digestion. The nature of the chemical effect appeared to limit the extent or degree to which the anaerobic microorganisms were able to metabolize the organic wastes and appeared to occur with the non-methanogenic species.

Ferric chloride addition to wastewater produced sludge that, when batch digested anaerobically, demonstrated reduced methane production, however the magnitude of the adverse effects did not increase with increasing iron concentrations up to 770 mg/L. The nature of the chemical effect appeared to occur with the non-methanogenic species.

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APPENDIX I Alkalinity Data for Alum Experiment R6

Table I.1 Alum Experiment R6

ALKALINITY DATA

	Day 0	Day 14	Day 28	Day 42	Day 56
CONTROL	990	1,390	1,450	1,480	1,480
II	960	1,440	1,460	1,490	1,470
IV	1,040	1,500	1,600	1,610	1,610
VI	1,080	1,650	1,680	1,720	1,710
VIII	1,120	1,700	1,820	1,840	1,830
CONTROL+	2,630	3,090	3,140	3,210	3,180
II+	2,630	3,070	3,190	3,190	3,170
IV+	2,670	3,240	3,300	3,270	3,310
VI+	2,700	3,310	3,370	3,400	3,360
VIII+	2,740	3,340	3,460	3,540	3,510

+ Treatment conditions with alkalinity supplement.

NOTE: All concentrations are mg/L as $CaCO_3$.

Alkalinity Increases in Alum Experiment R6

The observed alkalinity increases (difference in alkalinity levels between day 56 and day 0 from Table I.1) are presented below. Also shown below is the associated NH_3-N assuming the increased alkalinity originated from ammonia released from catabolism of organic nitrogen materials according to the equation (Gossett et al.,1978):

$$NH_3 + CO_2 \leftrightarrow HCO_3^- + NH_4^+.$$

NH_3-N values were calculated by multiplying the bicarbonate alkalinity concentrations by 14/50.

	ALKALINITY (mg/L as $CaCO_3$)	NH_3-N (mg/L)
CONTROL	490	137
II	510	143
IV	570	160
VI	630	176
VIII	710	199
CONTROL+	550	154
II+	540	151
IV+	640	179
VI+	660	185
VIII+	770	216

+ Treatment conditions with added alkalinity.

An approximate estimate of the nitrogen component in activated sludge without chemical addition is 5% by weight dry total solids (expressed as N)(EPA, 1979). This estimate decreases for activated sludge treated with alum due to the increased fixed solids (non-volitile) from $Al(OH)_3$, however the percent nitrogen component expressed as a ratio with the volatile solids should not change dramatically. An estimate of the nitrogen component (as N) in the culture bottles can be calculated as follows (assuming the sludge in the culture bottles are primarily activated sludge):

The %nitrogen component (expressed as a ratio with volatile solids) in the control is =

{5% * Total solids (mg/L)}/{Volatile solids (mg/L)} =
{5% * 2,810 mg/L}/{1,870 mg/L} ≅ 7.5%;
(see Table 5.9 for total solids and volatile solids concentrations).

Assuming the ratio 7.5% N/volatile solids remains consistent for all treatment conditions, it is possible to estimate the NH_3-N component (refer to next page):

	VS (mg/L)[1]	NH_3-N (mg/L)[2]
CONT.	1,870	140
II	1,880	141
IV	1,930	145
VI	1,940	145
VIII	1,960	147
CONT. +	1,870	140
II +	1,880	141
IV +	1,930	145
VI +	1,940	145
VIII +	1,960	147

[1] Volatile solids from Table 5.9.
[2] 7.5% * VS (mg/L).

+ Treatment conditions with added alkalinity.

By comparing the ammonia associated concentrations from the observed alkalinity increases with the theoretical ammonia nitrogen concentrations originating from degradation of volatile solids, the increases in alkalinity could reasonably be attributed to catabolism of organic nitrogen materials. Other constituents such as borates, phosphates, and silicates (if they are present) may contribute to measured alkalinity values (APHA, 1980) and could have accounted for some of the variability in the measured values.

APPENDIX II Bioassay Tests

Table II.1 Alum Experiment R1 % **METHANE PER g/L VOLATILE SOLIDS**

Al (mg/L)	**CONTROL** 22	I 24	II 39	III 52	IV 62	V 69	VI 78	VII 87	VIII 94	IX 110	(1)
Day 1	2.63	2.67	2.44	2.40	2.36	2.30	2.28	2.21	2.14	2.07	2.50
s.d.(2)	*0.12*	*0.04*	*0.02*	*0.05*	*0.05*	*0.02*	*0.05*	*0.10*	*0.04*	*0.05*	
Day 2	3.84	3.86	3.70	3.50	3.48	3.38	3.31	3.24	3.11	3.03	3.66
s.d.	*0.12*	*0.08*	*0.02*	*0.05*	*0.10*	*0.01*	*0.05*	*0.15*	*0.08*	*0.03*	
Day 4	4.68	4.64	4.47	4.28	4.22	4.14	4.02	3.93	3.79	3.70	4.51
s.d.	*0.07*	*0.12*	*0.06*	*0.06*	*0.15*	*0.05*	*0.06*	*0.08*	*0.06*	*0.03*	
Day 6	5.36	5.00	4.99	4.74	4.74	4.52	4.41	4.20	4.16	4.07	5.11
s.d.	*0.04*	*0.19*	*0.11*	*0.10*	*0.21*	*0.08*	*0.11*	*0.08*	*0.07*	*0.03*	
Day 9	5.88	5.76	5.62	5.24	5.23	5.14	5.08	4.89	4.71	4.67	5.65
s.d.	*0.07*	*0.11*	*0.06*	*0.10*	*0.22*	*0.09*	*0.03*	*0.15*	*0.64*	*0.07*	
Day 14	6.44	6.33	6.18	5.78	5.72	5.52	5.47	5.33	5.15	5.00	6.18
s.d.	*0.09*	*0.15*	*0.08*	*0.10*	*0.24*	*0.07*	*0.09*	*0.16*	*0.10*	*0.05*	
Day 21	7.35	7.19	6.94	6.52	6.56	6.24	5.97	5.83	5.63	5.30	7.02
s.d.	*0.09*	*0.19*	*0.13*	*0.16*	*0.26*	*0.16*	*0.03*	*0.22*	*0.08*	*0.13*	
Day 25	7.39	7.31	7.10	6.77	6.71	6.50	6.22	5.91	5.67	5.45	7.05
s.d.	*0.07*	*0.19*	*0.09*	*0.06*	*0.24*	*0.18*	*0.09*	*0.25*	*0.11*	*0.15*	
Day 32	7.74	7.69	7.41	7.03	6.97	6.73	6.61	6.29	5.91	5.65	7.46
s.d.	*0.07*	*0.15*	*0.06*	*0.14*	*0.22*	*0.16*	*0.07*	*0.19*	*0.08*	*0.06*	

(1) Low end of non-significant range for a 1-sided test ($P < 0.05$).
(2) Standard Deviation

Table II.1 Alum Experiment R1 (con't) % METHANE PER g/L VOLATILE SOLIDS

	CONTROL	I	II	III	IV	V	VI	VII	VIII	IX	
Al (mg/L)	22	24	39	52	62	69	78	87	94	110	(1)
Day 39	7.98	7.80	7.59	7.11	7.09	7.00	6.76	6.42	5.92	5.65	7.63
s.d.	*0.09*	*0.17*	*0.10*	*0.17*	*0.25*	*0.23*	*0.05*	*0.23*	*0.09*	*0.10*	
Day 45	8.33	8.14	7.88	7.40	7.54	7.35	7.12	6.77	6.07	5.73	7.95
s.d.	*0.02*	*0.13*	*0.07*	*0.10*	*0.34*	*0.14*	*0.11*	*0.21*	*0.15*	*0.24*	
Day 52	8.81	8.60	8.32	7.92	8.03	7.90	7.62	7.27	6.67	6.29	8.45
s.d.	*0.05*	*0.24*	*0.12*	*0.15*	*0.23*	*0.25*	*0.05*	*0.20*	*0.10*	*0.11*	
Day 57	8.81	8.58	8.32	7.97	7.99	7.88	7.61	7.33	6.79	6.29	8.47
s.d.	*0.01*	*0.31*	*0.02*	*0.19*	*0.26*	*0.08*	*0.03*	*0.18*	*0.05*	*0.03*	

Table II.2 Ferric Chloride Experiment R1 % METHANE PER g/L VOLATILE SOLIDS

Iron (mg/L)	CONTROL 9	I 31	II 56	III 120	IV 153	V 211	VI 246	VII 354	VIII 622	IX 770	(1)
Day 1	2.13	1.93	1.74	1.70	1.62	1.45	1.26	1.11	1.14	1.10	2.01
s.d.(2)	*0.05*	*0.06*	*0.02*	*0.04*	*0.03*	*0.08*	*0.05*	*0.05*	*0.03*	*0.12*	
Day 2	3.42	3.16	2.95	2.92	2.68	2.42	2.17	2.04	2.11	2.15	3.28
s.d.	*0.08*	*0.07*	*0.04*	*0.05*	*0.03*	*0.07*	*0.05*	*0.03*	*0.02*	*0.12*	
Day 4	4.29	4.24	3.91	3.95	3.57	3.20	2.89	2.75	2.81	2.84	4.08
s.d.	*0.25*	*0.10*	*0.06*	*0.10*	*0.05*	*0.04*	*0.05*	*0.03*	*0.04*	*0.07*	
Day 6	5.05	4.82	4.51	4.60	4.19	3.79	3.29	3.17	3.33	3.33	4.91
s.d.	*0.09*	*0.05*	*0.04*	*0.10*	*0.06*	*0.03*	*0.04*	*0.02*	*0.07*	*0.11*	
Day 9	5.83	5.62	5.23	5.39	5.02	4.60	3.92	3.78	4.00	4.09	5.65
s.d.	*0.03*	*0.09*	*0.12*	*0.12*	*0.13*	*0.05*	*0.06*	*0.01*	*0.05*	*0.09*	
Day 12	6.20	6.02	5.63	5.76	5.37	4.97	4.21	4.05	4.40	4.36	6.01
s.d.	*0.09*	*0.11*	*0.11*	*0.14*	*0.05*	*0.06*	*0.03*	*0.03*	*0.07*	*0.10*	
Day 15	6.53	6.31	5.93	6.15	5.84	5.46	4.64	4.51	4.86	4.89	6.30
s.d.	*0.04*	*0.06*	*0.10*	*0.18*	*0.22*	*0.06*	*0.04*	*0.03*	*0.01*	*0.11*	
Day 23	7.05	6.97	6.57	6.94	6.75	6.46	5.50	5.38	5.87	5.81	6.69
s.d.	*0.16*	*0.16*	*0.17*	*0.21*	*0.27*	*0.16*	*0.07*	*0.11*	*0.21*	*0.04*	
Day 30	7.49	7.09	6.68	7.03	7.24	6.80	6.02	5.93	6.45	6.57	6.94
s.d.	*0.18*	*0.17*	*0.33*	*0.53*	*0.28*	*0.18*	*0.19*	*0.20*	*0.17*	*0.12*	

(1) Low end of non-significant range for a 1-sided test ($P < 0.05$).
(2) Standard Deviation

Table II.2 Ferric Chloride Experiment R1 (con't) % METHANE PER g/L VOLATILE SOLIDS

Iron (mg/L)	CONTROL 9	I 31	II 56	III 120	IV 153	V 211	VI 246	VII 354	VIII 622	IX 770	(1)
Day 37	7.51	7.50	7.18	7.48	7.28	6.99	6.15	6.12	6.77	6.89	7.09
s.d.	*0.14*	*0.14*	*0.25*	*0.40*	*0.22*	*0.19*	*0.13*	*0.16*	*0.11*	*0.11*	
Day 43	7.88	7.97	7.49	7.50	7.75	7.44	6.59	6.57	7.15	7.25	7.32
s.d.	*0.19*	*0.37*	*0.17*	*0.48*	*0.24*	*0.22*	*0.29*	*0.23*	*0.14*	*0.10*	
Day 50	8.49	8.57	8.03	8.46	8.45	8.13	7.13	7.17	7.73	7.89	8.06
s.d.	*0.19*	*0.24*	*0.19*	*0.30*	*0.25*	*0.19*	*0.18*	*0.19*	*0.08*	*0.11*	
Day 55	8.33	8.47	7.99	8.41	8.19	7.96	7.24	7.16	7.62	7.89	7.94
s.d.	*0.22*	*0.18*	*0.19*	*0.26*	*0.21*	*0.20*	*0.12*	*0.15*	*0.12*	*0.16*	

Table II.3 Alum Experiment R2 % METHANE PER g/L VOLATILE SOLIDS

Al (mg/L)	CONTROL 20	0 19	I(a) 30	I(b) 30	II(a) 43	II(b) 44	III(a) 71	III(b) 78	IV 87	V 110	(1)
Day 1	3.16	3.18	2.97	2.91	2.62	2.63	2.36	2.51	2.60	2.60	2.94
s.d.(2)	*0.16*	*0.12*	*0.02*	*0.07*	*0.02*	*0.14*	*0.02*	*0.01*	*0.06*	*0.04*	
Day 2	4.81	4.51	4.64	4.68	4.47	4.45	4.23	4.25	4.53	4.46	4.60
s.d.	*0.12*	*0.14*	*0.04*	*0905*	*0.05*	*0.16*	*0.09*	*0.02*	*0.04*	*0.06*	
Day 4	5.48	5.36	5.27	5.27	5.17	5.09	5.02	5.05	5.32	5.33	5.29
s.d.	*0.19*	*0.21*	*0.04*	*0.05*	*0.07*	*0.12*	*0.14*	*0.07*	*0.05*	*0.09*	
Day 7	6.46	6.27	6.21	6.21	6.05	6.04	5.81	5.86	6.18	6.16	6.20
s.d.	*0.11*	*0.21*	*0.05*	*0.09*	*0.08*	*0.08*	*0.06*	*0.12*	*0.06*	*0.05*	
Day 11	7.31	7.06	6.86	6.88	6.63	6.64	6.40	6.40	6.70	6.59	7.12
s.d.	*0.11*	*0.23*	*0.03*	*0.13*	*0.08*	*0.15*	*0.07*	*0.08*	*0.01*	*0.06*	
Day 16	7.81	7.57	7.37	7.39	7.12	7.10	6.86	6.84	7.10	7.09	7.45
s.d.	*0.20*	*0.25*	*0.06*	*0.16*	*0.13*	*0.08*	*0.11*	*0.08*	*0.05*	*0.12*	
Day 23	7.98	7.74	7.41	7.48	6.83	6.87	6.92	6.85	7.20	7.10	5.52
s.d.	*0.20*	*0.22*	*0.03*	*0.08*	*0.34*	*0.36*	*0.03*	*0.11*	*0.07*	*0.11*	
Day 31	8.50	8.10	7.85	7.79	7.25	7.46	6.99	7.17	7.38	7.35	8.16
s.d.	*0.13*	*0.26*	*0.05*	*0.09*	*0.15*	*0.12*	*0.25*	*0.06*	*0.13*	*0.17*	
Day 37	9.06	8.78	8.17	8.38	7.69	7.87	7.50	7.65	7.99	7.85	8.74
s.d.	*0.15*	*0.19*	*0.09*	*0.21*	*0.11*	*0.11*	*0.14*	*0.29*	*0.08*	*0.18*	

(1) Low end of non-significant range for a 1-sided test ($P < 0.05$).
(2) Standard Deviation

Table II.3 Alum Experiment R2 (con't) % METHANE PER g/L VOLATILE SOLIDS

	CONTROL	0	I(a)	I(b)	II(a)	II(b)	III(a)	III(b)	IV	V	
Al (mg/L)	20	19	30	30	43	44	71	78	87	110	(1)
Day 45	8.94	8.60	8.19	8.05	7.83	7.83	7.50	7.43	7.88	7.87	8.73
s.d.	*0.13*	*0.19*	*0.09*	*0.23*	*0.08*	*0.20*	*0.04*	*0.05*	*0.06*	*0.05*	
Day 52	9.24	8.99	8.52	8.58	8.03	8.00	7.77	7.69	8.15	8.14	8.66
s.d.	*0.30*	*0.18*	*0.12*	*0.05*	*0.15*	*0.09*	*0.03*	*0.11*	*0.09*	*0.11*	
Day 59	9.57	9.22	8.69	8.75	8.35	8.37	8.16	8.07	8.44	8.41	9.19
s.d.	*0.20*	*0.31*	*0.05*	*0.08*	*0.08*	*0.13*	*0.10*	*0.13*	*0.13*	*0.05*	

Table II.4 Ferric Chloride Experiment R2 **% METHANE PER g/L VOLATILE SOLIDS**

Iron (mg/L)	**CONTROL** 7	0 7	II(a) 36	II(b) 32	III(a) 69	III(b) 75	(1)
Day 1	2.93	2.96	2.56	2.58	2.53	2.45	2.72
s.d.(2)	0.06	0.05	0.05	0.07	0.06	0.02	
Day 2	3.83	3.92	3.57	3.33	3.39	3.47	3.67
s.d.	0.09	0.08	0.08	0.12	0.08	0.01	
Day 5	5.51	5.69	4.94	4.88	4.98	4.99	5.38
s.d.	0.05	0.06	0.04	0.06	0.11	0.05	
Day 9	6.65	6.83	5.87	5.92	6.00	6.01	6.47
s.d.	0.09	0.04	0.01	0.03	0.09	0.14	
Day 14	7.39	7.30	6.44	6.21	6.32	6.47	7.03
s.d.	0.17	0.14	0.15	0.06	0.23	0.25	
Day 18	7.88	7.91	7.06	6.79	6.78	7.01	7.51
s.d.	0.19	0.15	0.16	0.05	0.15	0.23	
Day 21	7.96	8.04	7.12	6.92	6.88	7.10	7.62
s.d.	0.04	0.27	0.15	0.08	0.28	0.19	
Day 29	8.32	8.43	7.63	7.47	7.49	7.58	7.98
s.d.	0.12	0.20	0.20	0.06	0.24	0.24	
Day 35	9.14	9.22	8.26	8.17	8.19	8.57	8.39
s.d.	0.15	0.16	0.14	0.05	0.13	0.17	

(1) Low end of non-significant range for a 1-sided test ($P < 0.05$).
(2) Standard Deviation

Table II.4 Ferric Chloride Experiment R2 (con't) % METHANE PER g/L VOLATILE SOLIDS

	CONTROL	0	II(a)	II(b)	III(a)	III(b)	
Iron (mg/L)	7	7	36	32	69	75	(1)
Day 43	9.18	9.16	8.27	8.24	8.17	8.39	8.63
s.d.	*0.04*	*0.10*	*0.18*	*0.14*	*0.38*	*0.19*	
Day 50	9.61	9.66	8.58	8.52	8.54	8.81	9.20
s.d.	*0.03*	*0.19*	*0.07*	*0.10*	*0.35*	*0.14*	
Day 57	9.44	9.63	8.66	8.43	8.39	8.72	9.11
s.d.	*0.16*	*0.16*	*0.27*	*0.15*	*0.21*	*0.09*	

Table II.4 Ferric Chloride Experiment R2 (con't) % METHANE PER g/L VOLATILE SOLIDS

Iron (mg/L)	IV(a) 103	IV(b) 100	V(a) 161	V(b) 157	VI(a) 250	VI(b) 244	(1)
Day 1	2.54	2.63	2.63	2.66	2.56	2.48	2.72
s.d.(2)	0.10	0.09	0.12	0.13	0.15	0.13	
Day 2	3.44	3.50	3.35	3.44	3.24	3.24	3.67
s.d.	0.05	0.07	0.10	0.02	0.11	0.08	
Day 5	4.94	5.08	4.94	5.04	4.77	4.81	5.38
s.d.	0.03	0.07	0.06	0.04	0.01	0.07	
Day 9	6.02	6.07	6.02	6.01	5.77	5.79	6.47
s.d.	0.05	0.12	0.04	0.02	0.12	0.06	
Day 14	6.39	6.37	6.39	6.23	6.15	6.25	7.03
s.d.	0.11	0.19	0.05	0.17	0.09	0.20	
Day 18	6.94	6.81	6.57	6.75	6.58	6.86	7.51
s.d.	0.21	0.17	0.03	0.20	0.05	0.24	
Day 21	7.00	7.04	6.84	6.99	6.64	6.86	7.62
s.d.	0.04	0.25	0.09	0.05	0.08	0.21	
Day 29	7.54	7.56	7.52	7.55	7.25	7.30	7.98
s.d.	0.13	0.04	0.18	0.09	0.17	0.14	
Day 35	8.04	8.22	8.33	8.37	8.04	7.87	8.39
s.d.	0.69	0.45	0.21	0.10	0.30	0.49	

(1) Low end of non-significant range for a 1-sided test ($P \leq 0.05$).
(2) Standard Deviation

Table II.4 Ferric Chloride Experiment R2 (con't) % METHANE PER g/L VOLATILE SOLIDS

Iron (mg/L)	IV(a) 103	IV(b) 100	V(a) 161	V(b) 157	VI(a) 250	VI(b) 244	(1)
Day 43	8.22	8.45	8.07	8.36	8.10	8.11	8.63
s.d.	*0.38*	*0.14*	*0.44*	*0.16*	*0.14*	*0.14*	
Day 50	8.85	8.81	8.58	8.71	8.32	8.65	9.20
s.d.	*0.12*	*0.21*	*0.14*	*0.09*	*0.16*	*0.27*	
Day 57	8.78	8.72	8.71	8.85	8.44	8.54	9.11
s.d.	*0.07*	*0.27*	0.05	0.02	*0.21*	*0.13*	

Table II.5 Alum Experiment R3 % **METHANE PER g/L VOLATILE SOLIDS**

Al (mg/L)	**CONTROL** 20	II 33	IV 60	VI 85	VIII 108	(1)
Day 1	2.80	2.47	2.32	2.27	2.35	2.42
s.d.(2)	*0.15*	*0.09*	*0.33*	*0.22*	*0.02*	
Day 2	4.99	5.02	4.81	4.50	4.40	4.60
s.d.	*0.18*	*0.03*	*0.29*	*0.27*	*0.03*	
Day 4	5.89	6.02	5.76	5.38	5.31	5.49
s.d.	*0.14*	*0.07*	*0.30*	*0.30*	*0.07*	
Day 6	6.14	6.36	6.01	5.54	5.48	5.76
s.d.	*0.16*	*0.04*	*0.21*	*0.32*	*0.02*	
Day 11	7.05	7.03	6.53	6.23	6.09	6.64
s.d.	*0.18*	*0.04*	*0.30*	*0.28*	*0.03*	
Day 17	7.85	7.75	7.24	6.66	6.57	7.41
s.d.	*0.25*	*0.01*	*0.30*	*0.27*	*0.05*	
Day 25	8.30	8.03	7.43	6.98	6.65	7.80
s.d.	*0.28*	*0.03*	*0.36*	*0.29*	*0.06*	
Day 32	8.80	8.58	8.07	7.38	6.89	8.30
s.d.	*0.23*	*0.13*	*0.31*	*0.35*	*0.11*	
Day 39	8.77	8.63	8.07	7.43	7.03	8.29
s.d.	*0.30*	*0.04*	*0.28*	*0.33*	*0.08*	

(1) Low end of non-significant range for a 1-sided test ($P < 0.05$).
(2) Standard Deviation

Table II.5 Alum Experiment R3 (con't) **% METHANE PER g/L VOLATILE SOLIDS**

	CONTROL	**II**	**IV**	**VI**	**VIII**	
Al (mg/L)	20	33	60	85	108	(1)
Day 48	9.07	8.85	8.32	7.60	7.34	8.63
s.d.	*0.11*	*0.05*	*0.27*	*0.39*	*0.07*	
Day 54	9.37	9.11	8.48	7.84	7.53	8.96
s.d.	*0.31*	*0.05*	*0.16*	*0.28*	*0.05*	
Day 65	9.54	9.08	8.72	8.10	7.81	8.98
s.d.	*0.30*	*0.15*	*0.42*	*0.30*	*0.02*	

Table II.6 Alum Experiment R3 With Acetate Supplement % **METHANE PER g/L VOLATILE SOLIDS**

Al (mg/L)	**CONTROL** 20	**II** 33	**IV** 60	**VI** 85	**VIII** 108	**(1)**
Day 1	2.83	2.36	2.42	2.27	2.36	2.69
s.d.(2)	*0.04*	*0.05*	*0.01*	*0.11*	*0.08*	
Day 2	7.39	5.93	6.17	5.73	6.26	6.86
s.d.	*0.05*	*0.27*	*0.03*	*0.51*	*0.09*	
Day 4	10.25	9.15	8.95	8.90	9.10	10.06
s.d.	*0.05*	*0.10*	*0.10*	*0.11*	*0.08*	
Day 6	10.65	9.42	9.22	9.00	9.18	10.47
s.d.	*0.07*	*0.09*	*0.02*	*0.14*	*0.07*	
Day 11	11.10	9.69	9.23	9.36	9.42	10.61
s.d.	*0.05*	*0.15*	*0.49*	*0.08*	*0.16*	
Day 17	11.78	10.56	10.20	10.07	10.17	11.52
s.d.	*0.10*	*0.15*	*0.14*	*0.13*	*0.12*	
Day 25	12.15	10.70	10.40	10.04	9.94	11.82
s.d.	*0.10*	*0.21*	*0.06*	*0.08*	*0.26*	
Day 32	12.89	10.92	11.04	10.67	10.37	12.30
s.d.	*0.07*	*0.59*	*0.25*	*0.08*	*0.08*	
Day 39	12.90	11.33	11.23	10.80	10.66	12.61
s.d.	*0.18*	*0.10*	*0.08*	*0.14*	*0.17*	

(1) Low end of non-significant range for a 1-sided test ($P < 0.05$).
(2) Standard Deviation

Table II.6 Alum Experiment R3 With Acetate Supplement (con't) % METHANE PER g/L VOLATILE SOLIDS

	CONTROL	II	IV	VI	VIII	
Al (mg/L)	20	33	60	85	108	(1)
Day 48	12.93	11.41	11.29	11.24	10.38	12.21
s.d.	*0.16*	*0.17*	*0.03*	*0.17*	*0.75*	
Day 54	12.98	11.47	11.12	11.04	10.98	12.60
s.d.	*0.20*	*0.24*	*0.15*	*0.23*	*0.07*	
Day 65	13.19	11.70	11.38	11.41	11.28	12.84
s.d.	*0.16*	*0.29*	*0.13*	*0.11*	*0.11*	

Table II.7 Ferric Chloride Experiment R3 % METHANE PER g/L VOLATILE SOLIDS

	CONTROL	II	IV	V	VI	
Iron (mg/L)	8	36	98	176	364	(1)
Day 2	4.76	4.31	4.01	3.92	4.13	4.63
s.d.(2)	*0.05*	*0.08*	*0.01*	*0.08*	*0.07*	
Day 4	5.60	5.02	4.70	4.73	4.85	5.31
s.d.	*0.14*	*0.11*	*0.23*	*0.23*	*0.10*	
Day 6	6.24	5.55	5.28	5.17	5.37	6.03
s.d.	*0.13*	*0.08*	*0.02*	*0.12*	*0.12*	
Day 8	6.82	6.12	5.90	5.85	6.00	6.60
s.d.	*0.09*	*0.12*	*0.05*	*0.11*	*0.14*	
Day 10	7.32	6.53	6.30	5.99	6.39	6.88
s.d.	*0.12*	*0.11*	*0.15*	*0.42*	*0.10*	
Day 15	7.33	6.55	6.44	6.23	6.64	7.13
s.d.	*0.02*	*0.04*	*0.09*	*0.14*	*0.15*	
Day 18	8.04	7.16	7.03	6.79	7.13	7.86
s.d.	*0.10*	*0.06*	*0.09*	*0.09*	*0.09*	
Day 25	8.81	7.90	7.70	7.64	7.75	8.61
s.d.	*0.07*	*0.05*	*0.05*	*0.14*	*0.15*	
Day 32	8.95	7.96	7.81	7.65	7.98	8.78
s.d.	*0.14*	*0.10*	*0.01*	*0.09*	*0.02*	

(1) Low end of non-significant range for a 1-sided test ($P < 0.05$).
(2) Standard Deviation

Table II.7 Ferric Chloride Experiment R3 (con't) **% METHANE PER g/L VOLATILE SOLIDS**

Iron (mg/L)	**CONTROL** 8	**II** 36	**IV** 98	**V** 176	**VI** 364	(1)
Day 41	9.27	8.37	8.09	7.76	8.14	9.06
s.d.	*0.12*	*0.12*	*0.08*	*0.12*	*0.05*	
Day 47	9.63	8.64	8.75	8.33	8.61	9.32
s.d.	*0.17*	*0.16*	*0.17*	*0.15*	*0.13*	
Day 58	9.73	8.99	8.94	8.74	8.69	9.39
s.d.	*0.13*	*0.13*	*0.08*	*0.12*	*0.30*	

Table II.8 Ferric Chloride Experiment R3 With Acetate Supplement % **METHANE PER g/L VOLATILE SOLIDS**

Iron (mg/L)	**CONTROL** 8	II 36	IV 98	V 176	VI 364	(1)
Day 2	7.64	7.51	7.57	7.34	6.70	7.36
s.d.(2)	*0.16*	*0.12*	*0.07*	*0.18*	*0.15*	
Day 4	9.95	10.09	9.96	9.51	8.69	9.31
s.d.	*0.68*	*0.04*	*0.04*	*0.08*	*0.19*	
Day 6	10.91	10.62	10.40	10.13	9.29	10.67
s.d.	*0.11*	*0.06*	*0.13*	*0.12*	*0.14*	
Day 8	11.54	11.12	11.12	10.77	10.01	11.16
s.d.	*0.13*	*0.14*	*0.14*	*0.21*	*0.27*	
Day 10	11.38	11.19	11.33	10.70	10.14	
s.d.	*0.48*	*0.45*	*0.15*	*0.66*	*0.14*	
Day 15	11.45	11.30	11.23	10.86	10.17	11.21
s.d.	*0.07*	*0.11*	*0.02*	*0.11*	*0.20*	
Day 18	11.84	11.66	11.67	11.28	10.61	11.32
s.d.	*0.49*	*0.08*	*0.13*	*0.14*	*0.21*	
Day 25	13.09	12.60	12.47	12.26	11.44	12.60
s.d.	*0.33*	*0.05*	*0.14*	*0.17*	*0.37*	
Day 32	12.91	12.59	12.56	12.16	11.47	12.50
s.d.	*0.26*	*0.12*	*0.13*	*0.15*	*0.30*	

(1) Low end of non-significant range for a 1-sided test ($P < 0.05$).
(2) Standard Deviation

Table II.8 Ferric Chloride Experiment R3 With Acetate Supplement (con't) **% METHANE PER g/L VOLATILE SOLIDS**

	CONTROL	II	IV	V	VI	
Iron (mg/L)	8	36	98	176	364	(1)
Day 41	13.48	12.94	12.76	12.22	11.65	13.01
s.d.	*0.13*	*0.34*	*0.20*	*0.08*	*0.28*	
Day 47	13.38	13.19	13.27	12.50	11.98	13.06
s.d.	*0.23*	*0.08*	*0.17*	*0.16*	*0.10*	
Day 58	13.37	13.25	13.46	12.82	12.18	12.87
s.d.	*0.23*	*0.22*	*0.10*	*0.14*	*0.42*	

Table II.9 Alum Experiment R4 % METHANE PER g/L VOLATILE SOLIDS

Al (mg/L)	CONTROL 16	II 28	III 39	IV 56	V 67	VII 90	VIII 118	IX 144	(1)
Day 1	1.89	1.50	1.51	1.74	2.12	2.21	2.13	2.20	1.54
s.d.(2)	*0.04*	*0.09*	*0.03*	*0.09*	*0.11*	*0.18*	*0.34*	*0.24*	
Day 3	4.47	4.70	4.61	4.75	4.77	4.73	4.63	4.59	4.25
s.d.	*0.06*	*0.04*	*0.13*	*0.16*	*0.08*	*0.01*	*0.15*	*0.10*	
Day 5	5.56	5.70	5.65	5.80	5.70	5.68	5.58	5.55	5.31
s.d.	*0.10*	*0.09*	*0.11*	*0.18*	*0.11*	*0.05*	*0.17*	*0.09*	
Day 9	6.19	6.32	6.27	6.38	6.25	6.13	6.07	5.87	5.99
s.d.	*0.02*	*0.08*	*0.14*	*0.16*	*0.12*	*0.05*	*0.04*	*0.03*	
Day 12	6.60	6.66	6.61	6.59	6.39	6.24	6.06	5.92	6.41
s.d.	*0.06*	*0.13*	*0.13*	*0.15*	*0.06*	*0.03*	*0.06*	*0.01*	
Day 15	7.28	7.17	7.04	7.04	6.84	6.71	6.60	6.41	7.10
s.d.	*0.02*	*0.05*	*0.09*	*0.14*	*0.14*	*0.05*	*0.08*	*0.02*	
Day 22	7.95	7.82	7.79	7.65	7.57	7.15	6.68	6.83	7.64
s.d.	*0.10*	*0.16*	*0.15*	*0.19*	*0.10*	*0.08*	*0.26*	*0.08*	
Day 29	8.04	7.99	7.85	7.89	7.54	7.18	6.74	6.66	7.94
s.d.	*0.11*	*0.09*	*0.10*	*0.17*	*0.11*	*0.15*	*0.12*	*0.06*	
Day 38	8.73	8 37	8.22	8.42	7.88	7.58	7.34	6.99	8.36
s.d.	*0.15*	*0.08*	*0.20*	*0.38*	*0.10*	*0.12*	*0.04*	*0.09*	

(1) Low end of non-significant range for a 1-sided test ($P < 0.05$).
(2) Standard Deviation

Table II.8 Alum Experiment R4 (con't) % METHANE PER g/L VOLATILE SOLIDS

Al (mg/L)	CONTROL 16	II 28	III 39	IV 56	V 67	VII 90	VIII 118	IX 144	(1)
Day 44	9.15	8.79	8.66	8.84	8.34	8.03	7.58	7.48	8.82
s.d.	0.16	0.04	0.10	0.23	0.13	0.16	0.22	0.12	
Day 55	9.49	9.09	8.92	8.97	8.66	8.35	8.02	7.80	9.21
s.d.	0.15	0.11	0.06	0.22	0.10	0.18	0.05	0.08	
Day 60	10.21	9.68	9.63	9.59	9.25	8.92	8.57	8.36	9.93
s.d.	0.11	0.08	0.22	0.22	0.10	0.09	0.10	0.08	

Table II.10 Alum Experiment R5 % METHANE PER g/L VOLATILE SOLIDS

Al (mg/L)	CONTROL 647	II 733	III 733	IV 739	V 766	VII 772	VIII 778	IX 806	(1)
Day 2 *s.d.(2)*	2.82 *0.01*	2.75 *0.02*	2.77 *0.01*	2.76 *0.08*	2.75 *0.04*	2.81 *0.05*	2.81 *0.01*	2.73 *0.05*	2.74
Day 4 *s.d.*	3.47 *0.06*	3.38 *0.12*	3.36 *0.03*	3.40 *0.07*	3.39 *0.05*	3.40 *0.03*	3.49 *0.03*	3.37 *0.12*	3.31
Day 6 *s.d.*	3.74 *0.05*	3.67 *0.06*	3.63 *0.03*	3.68 *0.10*	3.64 *0.07*	3.65 *0.07*	3.70 *0.03*	3.62 *0.01*	3.62
Day 9 *s.d.*	4.08 *0.08*	3.90 *0.02*	3.91 *0.01*	3.96 *0.11*	3.86 *0.07*	3.79 *0.01*	3.86 *0.05*	3.77 *0.05*	3.95
Day 13 *s.d.*	4.19 *0.04*	4.09 *0.03*	4.05 *0.03*	4.10 *0.17*	4.08 *0.09*	4.06 *0.05*	4.09 *0.05*	3.98 *0.04*	4.04
Day 18 *s.d.*	4.34 *0.03*	4.11 *0.07*	4.16 *0.10*	4.21 *0.12*	4.17 *0.07*	4.19 *0.04*	4.19 *0.02*	4.13 *0.06*	4.19
Day 24 *s.d.*	4.35 *0.07*	4.20 *0.06*	4.27 *0.12*	4.32 *0.12*	4.25 *0.08*	4.30 *0.03*	4.29 *0.08*	4.22 *0.05*	4.18
Day 35 *s.d.*	4.51 *0.08*	4.27 *0.04*	4.34 *0.04*	4.56 *0.15*	4.31 *0.05*	4.34 *0.04*	4.42 *0.09*	4.28 *0.04*	4.35
Day 40 *s.d.*	4.57 *0.05*	4.34 *0.03*	4.44 *0.06*	4.47 *0.12*	4.40 *0.08*	4.41 *0.06*	4.53 *0.07*	4.37 *0.04*	4.43

(1) Low end of non-significant range for a 1-sided test ($P < 0.05$).
(2) Standard Deviation

Table II.10 Alum Experiment R5 (con't) % METHANE PER g/L VOLATILE SOLIDS

Al (mg/L)	CONTROL 647	II 733	III 733	IV 739	V 766	VII 772	VIII 778	IX 806	(1)
Day 46	4.70	4.51	4.54	4.60	4.55	4.54	4.66	4.47	4.57
s.d.	0.05	0.02	0.04	0.13	0.06	0.04	0.06	0.03	
Day 47	4.42	4.36	4.35	4.40	4.34	4.36	4.39	4.28	4.32
s.d.	0.03	0.04	0.03	0.11	0.03	0.01	0.03	0.03	
Day 54	4.34	4.27	4.26	4.30	4.26	4.27	4.33	4.31	4.21
s.d.	0.04	0.02	0.02	0.12	0.09	0.01	0.03	0.08	

Table II.11 Alum Experiment R6 % METHANE PER g/L VOLATILE SOLIDS

	CONTROL	II	IV	VI	VIII	
Al (mg/L)	10	21	47	80.	110	(1)
Day 2	5.08	5.01	4.92	4.98	4.67	4.50
s.d.(2)	0.04	0.23	0.20	0.14	0.26	
Day 4	6.25	6.04	5.82	6.04	5.73	5.88
s.d.	0.03	0.27	0.15	0.18	0.22	
Day 6	7.27	7.05	6.72	6.92	6.52	6.94
s.d.	0.07	0.19	0.08	0.20	0.21	
Day 8	7.65	7.38	7.00	7.13	6.79	7.35
s.d.	0.07	0.23	0.06	0.14	0.17	
Day 10	8.07	7.80	7.29	7.47	7.06	
s.d.	0.03	0.29	0.03	0.03	0.18	
Day 16	8.98	8.76	8.24	8.15	7.67	8.68
s.d.	0.09	0.19	0.03	0.16	0.21	
Day 20	9.23	9.01	8.62	8.23	7.69	9.00
s.d.	0.06	0.16	0.11	0.10	0.12	
Day 27	9.46	9.29	8.62	8.67	7.63	9.03
s.d.	0.01	0.17	0.15	0.25	0.33	
Day 33	10.40	10.17	9.57	9.61	8.66	10.03
s.d.	0.06	0.28	0.10	0.17	0.21	

(1) Low end of non-significant range for a 1-sided test ($P < 0.05$).
(2) Standard Deviation

Table II.11 Alum Experiment R6 (con't) % METHANE PER g/L VOLATILE SOLIDS

Al (mg/L)	CONTROL 10	II 21	IV 47	VI 80	VIII 110	(1)
Day 40	10.29	10.07	9.49	9.53	8.55	9.85
s.d.	*0.12*	*0.11*	*0.32*	*0.28*	*0.20*	
Day 47	10.53	10.36	9.76	9.63	8.78	10.23
s.d.	*0.11*	*0.11*	*0.15*	*0.15*	*0.20*	
Day 56	10.85	10.71	10.22	10.09	9.15	10.52
s.d.	*0.10*	*0.01*	*0.22*	*0.12*	*0.26*	

Table II.12 Alum Experiment R6 With Alkalinity Supplement % METHANE PER g/L VOLATILE SOLIDS

Al (mg/L)	CONTROL 10	II 21	IV 47	VI 80	VIII 110	(1)
Day 2	5.08	5.02	4.94	4.76	4.67	4.71
s.d.(2)	*0.16*	*0.10*	*0.11*	*0.27*	*0.23*	
Day 4	6.31	6.17	6.03	5.99	5.80	5.97
s.d.	*0.14*	*0.01*	*0.09*	*0.21*	*0.26*	
Day 6	7.37	6.99	6.77	6.68	6.59	7.08
s.d.	*0.20*	*0.06*	*0.09*	*0.16*	*0.16*	
Day 8	7.64	7.39	7.09	6.98	6.88	7.40
s.d.	*0.16*	*0.06*	*0.04*	*0.12*	*0.17*	
Day 10	8.18	7.79	7.41	7.21	7.03	7.82
s.d.	*0.17*	*0.12*	*0.12*	*0.25*	*0.19*	
Day 16	9.45	8.97	8.24	7.85	7.66	9.07
s.d.	*0.32*	*0.18*	*0.11*	*0.15*	*0.08*	
Day 20	9.34	8.89	8.32	7.88	7.63	8.95
s.d.	*0.26*	*0.12*	*0.11*	*0.10*	*0.28*	
Day 27	9.54	9.10	8.48	8.17	7.68	9.20
s.d.	*0.18*	*0.20*	*0.07*	*0.12*	*0.20*	
Day 33	10.52	10.07	9.50	9.08	8.57	10.23
s.d.	*0.19*	*0.14*	*0.15*	*0.15*	*0.03*	

(1) Low end of non-significant range for a 1-sided test ($P < 0.05$).
(2) Standard Deviation

Table II.12 Alum Experiment R6 With Alkalinity Supplement (con't) % METHANE PER g/L VOLATILE SOLIDS

	CONTROL	**II**	**IV**	**VI**	**VIII**	
Al (mg/L)	10	21	47	80	110	(1)
Day 40	10.48	9.85	9.35	8.99	8.38	10.12
s.d.	*0.21*	*0.14*	*0.09*	*0.12*	*0.27*	
Day 47	10.50	9.99	9.52	9.15	8.66	10.26
s.d.	*0.18*	*0.09*	*0.09*	*0.08*	*0.15*	
Day 56	10.78	10.27	9.34	9.18	8.89	10.33
s.d.	*0.08*	*0.18*	*0.33*	*0.27*	*0.18*	

APPENDIX III Methane Generation

Table III.1 Alum Experiment R1 **METHANE GENERATION**

		DAY 25		DAY 39		DAY 55	
	Al (mg/L)	**A**	**B**	**A**	**B**	**A**	**B**
CONT.	*22*	7.39	*100*	7.98	*100*	8.81	*100*
I	*24*	7.31	*99*	7.80	*98*	8.59	*98*
II	*39*	7.10	*96*	7.59	*95*	8.32	*97*
III	*52*	6.77	*92*	7.11	*89*	7.95	*90*
IV	*62*	6.71	*91*	7.09	*89*	8.01	*91*
V	*69*	6.50	*88*	7.00	*88*	7.89	*90*
VI	*78*	6.22	*84*	6.76	*85*	7.61	*86*
VII	*87*	5.91	*80*	6.42	*81*	7.30	*83*
VIII	*94*	5.67	*77*	5.92	*74*	6.73	*76*
IX	*110*	5.45	*74*	5.65	*71*	6.29	*71*

A = % methane per g/L volatile solids (from Table II.1).

B = Methane generation (% of control).

Table III.2 Ferric Chloride Experiment R1 **METHANE GENERATION**

	Fe (mg/L)	DAY 23		DAY 37		DAY 55	
		A	B	A	B	A	B
CONT.	9	7.05	100	7.51	100	8.33	100
I	31	6.97	99	7.50	100	8.47	102
II	56	6.57	93	7.18	96	7.99	96
III	120	6.94	98	7.48	100	8.41	101
IV	153	6.75	96	7.28	97	8.19	98
V	211	6.46	92	6.99	93	7.96	96
VI	246	5.50	78	6.15	82	7.24	87
VII	354	5.38	76	6.12	81	7.16	86
VIII	622	5.87	83	6.77	90	7.62	91
IX	770	5.81	82	6.89	92	7.89	95

A = % methane per g/L volatile solids (from Table II.2).

B = Methane generation (% of control).

Table III.3 Alum Experiment R2 **METHANE GENERATION**

		DAY 23		DAY 37		DAY 55	
	Al (mg/L)	**A**	**B**	**A**	**B**	**A**	**B**
CONT.	20	7.98	100	9.06	100	9.41	100
0	19	7.74	97	8.78	97	9.11	97
I(a)	30	7.41	93	8.17	90	8.60	91
I(b)	30	7.48	94	8.38	92	8.67	92
II(a)	43	6.83	86	7.69	85	8.19	87
II(b)	44	6.87	86	7.87	87	8.19	87
III(a)	71	6.92	87	7.50	83	7.97	85
III(b)	78	6.85	86	7.65	84	7.88	84
IV	87	7.20	90	7.99	88	8.30	88
V	110	7.10	89	7.85	87	8.28	88

A = % methane per g/L volatile solids (from Table II.3).

B = Methane generation (% of control).

Table III.4 Ferric Chloride Experiment R2 **METHANE GENERATION**

	Fe (mg/L)	DAY 29 A	DAY 29 B	DAY 43 A	DAY 43 B	DAY 55 A	DAY 55 B
CONT.	7	8.32	100	9.18	100	9.50	100
0	7	8.43	101	9.16	100	9.64	101
II(a)	36	7.63	92	8.27	90	8.64	91
II(b)	32	7.47	90	8.24	90	8.45	89
III(a)	69	7.49	90	8.17	89	8.40	88
III(b)	75	7.58	91	8.39	91	8.75	92
IV(a)	103	7.54	91	8.22	90	8.80	93
IV(b)	100	7.56	91	8.45	92	8.75	92
V(a)	161	7.52	90	8.07	88	8.67	91
V(b)	157	7.55	91	8.36	91	8.81	93
VI(a)	250	7.25	87	8.10	88	8.41	89
VI(b)	244	7.30	88	8.11	88	8.56	90

A = % methane per g/L volatile solids (from Table II.4).

B = Methane generation (% of control).

Table III.5 Alum Experiment R3 **METHANE GENERATION**

		DAY 25		DAY 39		DAY 55	
	Al (mg/L)	**A**	**B**	**A**	**B**	**A**	**B**
CONT.	20	8.30	100	8.77	100	9.46	100
II	33	8.03	97	8.63	98	9.10	96
IV	60	7.43	90	8.07	92	8.60	91
VI	85	6.98	84	7.43	85	7.97	84
VIII	108	6.65	80	7.03	80	7.67	81

A = % methane per g/L volatile solids (from Table II.5).

B = Methane generation (% of control).

Table III.6 Alum Experiment R3 With Acetate Supplement
METHANE GENERATION

		DAY 25		DAY 39		DAY 55	
	Al (mg/L)	A	B	A	B	A	B
CONT.	20	12.15	100	12.90	100	13.08	100
II	33	10.70	88	11.33	88	11.58	89
IV	60	10.40	86	11.23	87	11.25	86
VI	85	10.04	83	10.80	84	11.23	86
VIII	108	9.94	82	10.66	83	11.13	85

A = % methane per g/L volatile solids (from Table II.6).

B = Methane generation (% of control).

Table III.7 Ferric Chloride Experiment R3 **METHANE GENERATION**

		DAY 25		DAY 41		DAY 55	
	Fe (mg/L)	**A**	**B**	**A**	**B**	**A**	**B**
CONT.	8	8.81	100	9.27	100	9.70	100
II	36	7.90	90	8.37	90	8.89	92
IV	98	7.70	87	8.09	87	8.88	92
V	176	7.64	87	7.76	84	8.63	89
VI	364	7.75	88	8.14	88	8.67	89

A = % methane per g/L volatile solids (from Table II.7).

B = Methane generation (% of control).

Table III.8 Ferric Chloride Experiment R3 With Acetate Supplement
METHANE GENERATION

	Fe (mg/L)	DAY 25 A	DAY 25 B	DAY41 A	DAY41 B	DAY 55 A	DAY 55 B
CONT.	8	13.09	100	13.48	100	13.38	100
II	36	12.60	96	12.94	96	13.24	99
IV	98	12.47	95	12.76	95	13.40	100
V	176	12.26	94	12.22	91	12.72	95
VI	364	11.44	87	11.65	86	12.12	91

A = % methane per g/L volatile solids (from Table II.8).

B = Methane generation (% of control).

Table III.9 Alum Experiment R4 **METHANE GENERATION**

	Al (mg/L)	DAY 22 A	DAY 22 B	DAY 38 A	DAY 38 B	DAY 55 A	DAY 55 B
CONT.	16	7.95	100	8.73	100	9.49	100
II	28	7.82	98	8.37	96	9.09	96
III	39	7.79	98	8.22	94	8.92	94
IV	56	7.65	96	8.42	96	8.97	95
V	67	7.57	95	7.88	90	8.66	91
VII	90	7.15	90	7.58	87	8.35	88
VIII	118	6.68	84	7.34	84	8.02	85
IX	144	6.83	86	6.99	80	7.80	82

A = % methane per g/L volatile solids (from Table II.9).

B = Methane generation (% of control).

Table III.10 Alum Experiment R6 **METHANE GENERATION**

		DAY 27		DAY 40		DAY 55	
	Al (mg/L)	**A**	**B**	**A**	**B**	**A**	**B**
CONT.	*10*	*9.46*	*100*	*10.29*	*100*	*10.85*	*100*
II	*21*	*9.29*	*98*	*10.07*	*98*	*10.71*	*99*
IV	*47*	*8.62*	*91*	*9.49*	*92*	*10.22*	*94*
VI	*80*	*8.67*	*92*	*9.53*	*93*	*10.09*	*93*
VIII	*110*	*7.63*	*81*	*8.55*	*83*	*9.15*	*84*

A = % methane per g/L volatile solids (from Table II.11).

B = Methane generation (% of control).

Table III.11 Alum Experiment R6 With Alkalinity Supplement
METHANE GENERATION

	Al (mg/L)	DAY 27 A	DAY 27 B	DAY 40 A	DAY 40 B	DAY 55 A	DAY 55 B
CONT.	10	9.54	100	10.48	100	10.78	100
II	21	9.10	95	9.85	94	10.27	95
IV	47	8.48	89	9.35	89	9.34	87
VI	80	8.17	86	8.99	86	9.18	85
VIII	110	7.68	81	8.38	80	8.89	82

A = % methane per g/L volatile solids (from Table II.12).

B = Methane generation (% of control).

APPENDIX IV Test For Significance on Coagulant Dose - Methane Response Relationship

The regression equation is represented by:

$Y = \beta_0 + \beta_1 X$; where

Y = methane generation (% of control);

X = coagulant dose (Al^{3+} or Fe^{3+}) mg/L;

β_1 = slope of the regression equation; and

β_0 = value of Y at $X = 0$.

Significance test for $\beta_1 = 0$ versus $\beta_1 \neq 0$ using F^* statistic for the analysis of variance (ANOVA) approach ($P < 0.05$): $F^* = MSR/MSE$; where

MSR = mean square due to regression; and

MSE = mean square due to error.

Hypothesis test:

Null hypothesis; C_1: $\beta_1 = 0$,

Alternative hypothesis; C_2: $\beta_1 \neq 0$.

Decision rule:

If $F^* \leq F(.95 ; \nu_1 , \nu_2)$ conclude C_1;

If $F^* > F(.95 ; \nu_1 , \nu_2)$ conclude C_2; where

ν_1 = degrees of freedom for regression mean square; and

ν_2 = degrees of freedom for error mean square.

Alum Experiment R1

Regression Equation:

$$Y = 106.9 - 0.305X$$

ANOVA TABLE:

	df	*ss*	*ms*
REGRESSION	*1*	*733.48*	*MSR = 733.48*
ERROR	*8*	*23.02*	*MSE = 2.88*
TOTAL	*9*	*756.50*	

df = degrees freedom

ss = sum of squares

ms = mean square = *ss/df*

Significance Test:

$F^* = 733.48/2.88 = 254.68$

$F(.95;\ 1,\ 8) = 5.32$

Decision:

Since $F^* > F$ conclude C_2 ($\beta_1 \neq 0$) at the $P < 0.05$ significance level.

Ferric Chloride Experiment R1

Regression Equation:

$$Y = 96.9 - 0.016X$$

ANOVA TABLE:

	df	ss	ms
REGRESSION	1	154.34	MSR = 154.34
ERROR	8	311.76	MSE = 38.97
TOTAL	9	466.10	

df = degrees freedom

ss = sum of squares

ms = mean square = ss/df

Significance Test:

$F^* = 154.34/38.97 = 3.96$

$F(.95; 1, 8) = 5.32$

Decision:

Since $F^* \le F$ conclude C_1 $(\beta_1 = 0)$ at the $P < 0.05$ significance level.

Alum Experiment R2

Regression Equation:

$$Y = 95.9 - 0.108X$$

ANOVA TABLE:

	df	*ss*	*ms*
REGRESSION	*1*	*103.46*	*MSR = 103.46*
ERROR	*8*	*135.44*	*MSE = 16.93*
TOTAL	*9*	*238.90*	

df = degrees freedom

ss = sum of squares

ms = mean square = *ss/df*

Significance Test:

$$F^* = 103.46/16.93 = 6.11$$

$$F(.95;\ 1,\ 8) = 5.32$$

Decision:

Since $F^* > F$ conclude C_2 ($\beta_1 \neq 0$) at the $P < 0.05$ significance level.

Ferric Chloride Experiment R2

Regression Equation:

$$Y = 95.2 - 0.031X$$

ANOVA TABLE:

	df	*ss*	*ms*
REGRESSION	*1*	*75.39*	*MSR = 75.39*
ERROR	*10*	*114.61*	*MSE = 11.46*
TOTAL	*11*	*190.00*	

df = degrees freedom

ss = sum of squares

ms = mean square = *ss/df*

Significance Test:

$F^* = 75.39/11.46 = 6.58$

$F(.95; 1, 8) = 4.96$

Decision:

Since $F^* > F$ conclude C_1 ($\beta_1 \neq 0$) at the $P < 0.05$ significance level.

Alum Experiment R3

Regression Equation:

$$Y = 104.6 - 0.233X$$

ANOVA TABLE:

	df	*SS*	*ms*
REGRESSION	*1*	*284.11*	*MSR = 284.11*
ERROR	*3*	*1.09*	*MSE = 0.36*
TOTAL	*4*	*285.20*	

df = degrees freedom

SS = sum of squares

ms = mean square = *SS/df*

Significance Test:

$$F^{*} = 284.11/0.36 = 789.19$$

$$F(.95;\ 1,\ 3) = 10.1$$

Decision:

Since $F^{*} > F$ conclude C_2 ($\beta_1 \neq 0$) at the $P < 0.05$ significance level.

Alum Experiment R3 With Acetate Supplement

Regression Equation:

$$Y = 97.8 - 0.156X$$

ANOVA TABLE:

	df	*ss*	*ms*
REGRESSION	*1*	*128.43*	*MSR = 128.43*
ERROR	*3*	*60.37*	*MSE = 20.12*
TOTAL	*4*	*188.80*	

df = degrees freedom

ss = sum of squares

ms = mean square = *ss/df*

Significance Test:

$F^* = 128.43/20.12 = 6.38$

$F(.95;\ 1,\ 3) = 10.1$

Decision:

Since $F^* \leq F$ conclude C_1 ($\beta_1 = 0$) at the $P < 0.05$ significance level.

Ferric Chloride Experiment R3

Regression Equation:

$$Y = 94.2 - 0.024X$$

ANOVA TABLE:

	df	*ss*	*ms*
REGRESSION	*1*	*45.28*	*MSR = 45.28*
ERROR	*3*	*64.72*	*MSE = 21.57*
TOTAL	*4*	*110.00*	

df = degrees freedom

ss = sum of squares

ms = mean square = *ss/df*

Significance Test:

$F^* = 45.28/21.57 = 2.10$

$F(.95;\ 1,\ 3) = 10.1$

Decision:

Since $F^* \le F$ conclude C_1 ($\beta_1 = 0$) at the $P < 0.05$ significance level.

Ferric Chloride Experiment R3 With Acetate Supplement

Regression Equation:

$$Y = 99.3 - 0.032X$$

ANOVA TABLE:

	df	*ss*	*ms*
REGRESSION	*1*	*82.525*	*MSR = 82.525*
ERROR	*3*	*3.475*	*MSE = 1.158*
TOTAL	*4*	*86.000*	

df = degrees freedom

ss = sum of squares

ms = mean square = *ss/df*

Significance Test:

$F^* = 82.525/1.158 = 71,27$

$F(.95;\ 1,\ 3) = 10.1$

Decision:

Since F^* > F conclude C_2 ($\beta_1 \neq 0$) at the $P < 0.05$ significance level.

Alum Experiment R4

Regression Equation:

$$Y = 101.2 - 0.136X$$

ANOVA TABLE:

	df	*ss*	*ms*
REGRESSION	*1*	*259.42*	*MSR = 259.42*
ERROR	*6*	*8.08*	*MSE = 1.35*
TOTAL	*7*	*267.50*	

df = degrees freedom

ss = sum of squares

ms = mean square = *ss/df*

Significance Test:

$F^* = 259.42/1.35 = 192.16$

$F(.95;\ 1,\ 6) = 5.99$

Decision:

Since $F^* > F$ conclude C_2 ($\beta_1 \neq 0$) at the $P < 0.05$ significance level.

Alum Experiment R6

Regression Equation:

$$Y = 101.2 - 0.153X$$

ANOVA TABLE:

	df	*ss*	*ms*
REGRESSION	*1*	*160.74*	*MSR = 160.74*
ERROR	*3*	*15.26*	*MSE = 5.09*
TOTAL	*4*	*176.00*	

df = degrees freedom

ss = sum of squares

ms = mean square = *ss/df*

Significance Test:

$F^* = 160.74/5.09 = 31.58$

$F(.95; 1, 3) = 10.1$

Decision:

Since $F^* > F$ conclude C_2 ($\beta_1 \neq 0$) at the $P < 0.05$ significance level.

Alum Experiment R6 With Alkalinity Supplement

Regression Equation:

$$Y = 99.3 - 0.174X$$

ANOVA TABLE:

	df	ss	ms
REGRESSION	1	208.80	MSR = 208.80
ERROR	3	17.20	MSE = 5.73
TOTAL	4	226.00	

df = degrees freedom

ss = sum of squares

ms = mean square = ss/df

Significance Test:

$F^* = 208.80/5.73 = 36.44$

$F(.95;\ 1,\ 3) = 10.1$

Decision:

Since $F^* > F$ conclude C_2 ($\beta_1 \neq 0$) at the $P < 0.05$ significance level.

APPENDIX V Sulphates as a Precursor to Soluble Sulphide Toxicity

The effects of sulphide (S^{2-}) on anaerobic digestion with respect to the alum bioassays are discussed. Lawrence et al. (1964) indicates that soluble sulphide concentrations greater than 200 mg/L exert severe toxic effects upon the anaerobic digestion process (i.e. complete cessation of gas production), while concentrations less than 200 mg/L do not significantly affect the process. McCarty (1964b) indicates that soluble sulphide concentrations greater than 100 mg/L can cause moderate inhibition in anaerobic digestion (see Table 3.3).

Sulphides in anaerobic digesters can occur from (Lawrence et al.,1964);

1. sulphides present in the raw waste;
2. anaerobic protein degradation; and
3. sulphates and other sulpher containing inorganic compounds.

The first two sources are generally small.

The highest dosage of alum added to activated sludge was 2,000 mg to 2 L (equivalent dosage of 1,000 mg/L; see Table 4.1). The reactions of interest of alum with phosphates and alkalinity are (section 3.1.1):

$Al_2(SO_4)_3 \cdot 18H_2O + 2PO_4^{3-} \leftrightarrow 2AlPO_4\downarrow + 3SO_4^{2-} + 18H_2O$; and

$$Al_2(SO_4)_3 \cdot 18H_2O + 6HCO_3^- \leftrightarrow 2Al(OH)_3\downarrow + 3SO_4^{2-} + 6CO_2\uparrow + 18\ H_2O.$$

Three moles of sulphate (SO_4^{2-}) are generated (assuming complete conversion) from each mole of aluminum sulphate (from both reactions). The concentration of sulphates in the activated sludge sample receiving an equivalent alum dosage of 1,000 mg/L is (assuming complete conversion):

{(1,000 mg/L)/(g - mole weight of alum)} * 3 =
{(1,000 mg/L)/((666.42 g/mole)(1,000 mg/g))} * 3
= 0.0045 moles/L.

The reduction of sulphates to sulphide under anaerobic conditions may be expressed by the following equation (Lawrence et al., 1964):

$8H^+ + 8e^- + SO_4^{2-} \rightarrow$(sulphate-reducing bacteria)$\rightarrow S^{2-} + 4H_2O$.

Anaerobic protein decomposition will also contribute to sulphide concentrations:

organic matter $\rightarrow$(anaerobic bacteria)$\rightarrow S^{2-} + H_2O + CO_2$.

This source of sulphide contribution can be neglected because, as mentioned previously, it is small.

One mole of sulphide will be produced for each mole of sulphate reduced under anaerobic conditions assuming complete conversion. The concentration of sulphide in a 30 mL liquid volume of the alum precipitated sludge (equivalent alum dosage 1,000 mg/L) is:

(0.0045 moles/L) * (g - mole weight of S^{2-}) =
(0.0045 moles/L) * (32 g/mole)(1,000 mg/g) = 144 mg/L.

The concentration of sulphide in the serum bottle will actually be less. Thirty mL of the alum precipitated activated sludge (equivalent sulphide concentration of 144 mg/L) was diluted with 20 mL of other constituents

(50 mL total liquid volume; see section 4.3). The sulphide concentration in a 50 mL liquid volume sludge sample becomes:

$$(144\ \text{mg/L})(0.03\ \text{L})/(0.05\ \text{L}) = 86\ \text{mg/L}.$$

The 86 mg/L of sulphide in the sludge samples will be distributed in several forms (Lawrence et al., 1964):

1. as a soluble sulphide (S^{2-});
2. some will form a weak acid which ionizes in aqueous solutions, depending on the pH:
   $H_2S\ (\text{aqueous}) \leftrightarrow H^+ + HS^-$;
3. some will be converted to H_2S and be present in the headspace gas; and
4. the remainder will be in an insoluble form (non-toxic) due to heavy metal cationic precipitation (similar to discussion in section 3.2.2.4).

APPENDIX VI Acetate Conversion to Methane

Mass balances on the conversion of organic carbon to CH_4 and CO_2 are based upon the stoichiometry of the Buswell equation (Healy and Young, 1979):

$$C_nH_xO_y + (n - (x/4) - (y/2))H_2O \rightarrow$$

$$((n/2) - (x/8) + (y/4))CO_2 + ((n/2) + (x/8) - (y/4)CH_4.$$

Acetate is represented by CH_3COOH or $C_2H_4O_2$. The Buswell equation for acetate conversion is represented by:

$$C_2H_4O_2 \rightarrow CO_2 + CH_4.$$

A 0.5 mL liquid volume of acetate solution (75 mg/mL) was added to each serum bottle:

$$\# \text{ moles acetate} = (0.5 \text{ mL} * 75 \text{ mg/mL})/(60{,}000 \text{ mg/mole})$$

$$= 0.000625.$$

One mole of CH_4 is produced from each mole of $C_2H_4O_2$ (assuming complete conversion) from the Buswell equation for acetate conversion, therefore:

$$\# \text{ moles } CH_4 \text{ produced} = 0.000625 \text{ moles.}$$

At STP (standard temperature and pressure) 1 mole of CH_4 occupies a volume of 22.4 L. At STP 0.000625 moles of CH_4 occupies a volume of:

$$0.000625 \text{ moles} * 22.4 \text{ L/mole} = 0.014 \text{ L.}$$

Summary: The physical conditions for the CH_4 produced from acetate conversion only in the serum bottle at STP are:

$T = 0$ °C (273 °K);

$P = 1$ atm;

$V = 0.014$ L; and

$n = 0.000625$ moles.

To determine the volume the methane occupies under experimental conditions in the serum bottles, the pressure must be known. An analysis will be performed on data for the last day of Alum Experiment R3 (day 65) to determine the pressure in the serum bottles.

Experimental conditions:

T = room temperature = 20 °C (293 °K);

P = ?;

V = ?; and

n = 0.000625 moles.

The pressure (P) is due to the partial pressures from:

1. CH_4 production from acetate conversion;
2. CH_4 production from degradation of volatile solids;
3. other gas production (CO_2, N_2, H_2S, etc); and
4. presence of the 30% - 70% CO_2 - N_2 gas added during setup of the experiment (section 4.3).

Methane Production From Acetate and VS Destruction

	VS (g/L) (1)	CH_4/VS (%/g/L) Day 65 (2)	CH_4 (%) (3)	CH_4 (mL) (4)
CONT.	2.11	13.19	27.8	41.4
II	2.66	11.70	31.1	48.5
IV	2.65	11.38	30.2	46.5
VI	2.60	11.41	29.7	45.4
VIII	2.44	11.28	27.5	40.8

(1) - Taken from Table 5.5.

(2) - Taken from Table II.6.

(3) - (1)*(2).

(4) - Equation presented in section 4.4.3 was adapted to determine the volumes (see below).

$\%CH_4 = (a/(a + 107.5))\cdot 100$; where
a = volume (mL) of CH_4.

Volume = $(107.5(\%CH_4/100))/(1 - (\%CH_4/100))$; where

107.5 represents the available volume in the serum bottle headspace =
total volume (158 mL) - liquid volume (50.5 mL).

It can be assumed the volumes in (4) have been determined at atmospheric pressure because when making up methane standards (section 4.4.3), the purified methane added to the standard bottles are at "atmospheric pressure" in the syringe just prior to injecting to the standard bottle.

Other Gas Production

Approximately 70% of total gas production in anaerobic digestion is in the form of methane (section 3.2). The remaining 30% constitutes other gases. Hsu and Pipes (1973) reported that the compostion of digester gas (e.g. CH_4 and CO_2) did not change very much during anaerobic digestion of primary and waste activated sludge containing preformed $Al(OH)_3$ floc. This was observed for aluminum concentrations from 0 to 1,549 mg/L Al. Therefore the volumes of the other gases (as a total) can be estimated from methane production:

$(30\%/70\%) * CH_4$ volumes $\cong 40\% * CH_4$ volumes.

Estimated volumes of other gases are:

CONT. - 0.4 * 41.4 = 16.6 mL.
II - 0.4 * 48.5 = 19.4 mL.
VI - 0.4 * 46.5 = 18.6 mL.
VI - 0.4 * 45.4 = 18.2 mL.
VIII - 0.4 * 40.8 = 16.3 mL.

Volumes are determined at atmospheric pressure (because they are a ratio of the CH_4 volumes).

30%-70% Carbon Dioxide Nitrogen Mixture

Volume CO_2 in the serum bottle headspace = 0.3 * 107.5
= 32.3 mL.

Volume N_2 in the serum bottle headspace = 0.7 * 107.5
= 75.2 mL.

Volumes are assumed to be determined at atmospheric pressure (conditions that existed during setup of the experiment in section 4.3).

Determination of Number of Moles of Each Gas

The Ideal Gas Law equation will be used to determine the number of moles of each gas:

$$PV = nRT,$$ where

P = atmospheric pressure (assume = 1) atm;
V = volume of respective gas L;
n = # of moles;
R = 0.082057 L-atm/mole-°K; and
T = room temperature (assume = 20 °C) = 293 °K.

CH_4 production - # moles:

CONT. - 0.00172 moles;
II - 0.00202 moles;
IV - 0.00193 moles;
VI - 0.00189 moles; and
VIII - 0.00170 moles.

Other gas production - # moles:

CONT. - 0.000691 moles;
II - 0.000807 moles;
IV - 0.000774 moles;
VI - 0.000757 moles; and
VIII - 0.000678 moles.

CO_2 - N_2 mixture - # moles:

CO_2 - 0.00134 moles; and
N_2 - 0.00313 moles.

Total Pressure in the Serum Bottles From All Gases

$P = nRT/V$, where

n = # of moles of respective gas;
R = 0.082057 L-atm/mole-°K;
T = 293 °K; and
V = serum bottle headspace = 107.5 mL = 0.1075 L.

	CH_4 P (atm)	OTHER GAS - P (atm)	CO_2 P (atm)	N_2 P (atm)	TOTAL P (atm)
CONT.	0.3847	0.1545	0.3000	0.7000	1.54
II	0.4518	0.1805	0.3000	0.7000	1.63
IV	0.4317	0.1731	0.3000	0.7000	1.60
VI	0.4227	0.1693	0.3000	0.7000	1.59
VIII	0.3802	0.1516	0.3000	0.7000	1.53

Volume of Methane Produced From Acetate Conversion

The volume of methane produced from acetate conversion alone in the serum bottles (under experimental conditions) can be determined as follows -

at STP (previously calculated):

P = 1 atm;

T = 273 °K; and

V = 0.014 L.

at experimental conditions:

P = total pressures calculated (atm);

T = 20 °C = 293 °K; and

V = ? (L).

$$(PV/T) \text{ at STP} = (PV/T) \text{ at EXP.}$$

	TOTAL P (atm)	CALCULATED VOL. CH_4 (mL)
CONT.	1.54	9.8
II	1.63	9.2
IV	1.60	9.4
VI	1.59	9.5
VIII	1.53	9.8

The CH_4 volumes are calculated at experimental conditions (i.e. T = 20 °C).

Measured Methane Volumes From Acetate Conversion

Methane volumes are determined from the formula (as before):

Volume = $(107.5(\%CH_4/100))/(1 - (\%CH_4/100))$.

	MEASURED CH_4/VS (%/g/L) (1)	VS (g/L) (2)	CH_4 (%) (3)	VOL. CH_4 (mL)
CONT.	3.65	2.11	7.70	9.0
II	2.62	2.66	6.97	8.1
IV	2.66	2.65	7.05	8.2
VI	3.31	2.60	8.61	10.1
VIII	3.47	2.44	8.47	9.9

(1) Measured differences in $\%CH_4$ per g/L VS for day 65 from Table II.5 and Table II.6.
(2) Taken from Table 5.5.
(3) = (1)*(2).

The measured CH_4 volumes (mL) are compared to the calculated volumes for acetate conversion from the acetate supplement below (at experimental conditions):

ALUM EXP. R3	Al (mg/L)	MEASURED VOLUME CH_4 (mL)	CALCULATED VOLUME CH_4 (mL)
CONT.	20	9.0	9.8
II	33	8.1	9.2
IV	60	8.2	9.4
VI	85	10.1	9.5
VIII	108	9.9	9.8

The calculated volumes are actually over estimated because the analysis was performed assuming all the CH_4 (from conversion of the acetate supplement) was present in the serum bottle headspace (107.5 mL volume). In fact, some of the CH_4 will be dissolved in the liquid sample depending on the pressure.

Similarly, an analysis can be performed on $FeCl_3$ Experiment R3 (day 58) to compare the measured and calculated CH_4 volumes produced from conversion of the acetate supplement (refer to next page).

$FeCl_3$ EXP. R3	Fe (mg/L)	MEASURED VOLUME CH_4 (mL)	CALCULATED VOLUME CH_4 (mL)
CONT.	8	8.5	10.1
II	36	9.8	10.1
IV	98	10.4	10.0
VI	176	9.7	10.1
VIII	364	10.0	9.5